INHERITANCE OF NODULATION AND ITS ASSOCIATION WITH GENES CONTROLLING TESTA COLOR IN *Arachis hypogaea* L.

BY

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A DISSERTATION PRESENTED TO THE GRADUATE COUNCIL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1983

Dedicated to my parents,

Robert and Rosemary Dashiell

ACKNOWLEDGMENTS

The author is sincerely grateful for the advice, guidance, and encouragement given to him by the chairman of the supervisory committee, Dr. D. W. Gorbet. Sincere appreciation is also extended to the cochairman of the supervisory committee, Dr. A. J. Norden, for the advice and guidance he provided when I was in Gainesville. Sincere thanks are also extended to other members of my committee, Drs. E. S. Horner, D. H. Hubbell, and D. W. Dickson, whose doors were always open when I needed advice or assistance.

Special thanks are due to Wayne Branch, Charles Bryant, Mary Chambliss, Harold Hewett, Willis Lipford, and Stanley Slay for their technical assistance in the field and laboratory.

I am also grateful to my parents, Robert and Rosemary Dashiell, and my sister, Karla Dashiell, for hand shelling thousands of peanuts for me during their Christmas vacations in 1980 and 1981, and for their encouragement throughout this study.

Thanks are also given to Patricia French for typing this manuscript.

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April 1983

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A study was conducted on peanut (Arachis hypogaea L.) to determine the inheritance of nodulation and its association with genes controlling testa color. A diallel cross was made using M4-2, a non-nodulating line, and three nodulating peanut lines, PI 262090, UF 487A, and 'Florunner.' Selected F_1 plants were backcrossed to M4-2 or PI 262090. The F_1, F_2, F_3, F_1BC_1, and F_2BC_1 generations were field grown at the University of Florida Agricultural Research Center at Marianna, Florida. Nodulation classifications were determined by observing plants and rating roots on each plant from 0 (no nodules) through 5 (abundant nodules). Pod samples were taken and testa color was evaluated. These data were analyzed by chi-square test for goodness-of-fit to the proposed model.

The results indicate that inheritance of nodulation is controlled at two loci, $\underline{N_1}$ and $\underline{N_2}$. The non-nodulating genotype (M4-2) is $\underline{n_1n_1n_2n_2}$ and all other genotypes have normal nodulation, except $\underline{n_1n_1N_2n_2}$,

which had few nodules when the parental male gamete was n_1n_2. The locus controlling testa variegation, V, was found to be linked to N_1 with an average crossover rate of about 10%. Testa variegation color is controlled at one locus. The allele causing white variegation, Wv, is dominant to the allele causing tinted variegation, wv. The N_2 and Wv loci segregated independently. The R_2 locus, which controls red and pink testa color, segregated independently from the V locus. It was also determined that the N_1, N_2, and R_2 loci segregate independently.

In another study, two non-nodulating peanut lines, PI 445923 and PI 445924, were crossed with M4-2, PI 262090, UF 487A, and Florunner. Data were collected from the F_1 and F_2 generations for nodulation and testa color. The results do not fully support the model for inheritance of nodulation described in the first study. The allele causing purple testa color, P, appeared to be dominant to pink and red. There also appeared to be a duplicate locus of R_2 which was designated R_3. The following groups of loci were found to segregate independently (R_2, V), (P, R_2, R_3, V), (V, N_2), (R_2, N_2), (P, N_1, N_2), and (P, R_2, R_3, N_2).

CHAPTER 1
INTRODUCTION

Crops that fix their own N_2 have an inherent advantage over crops which cannot. This advantage may become even more importan as the cost of N fertilizer, which is derived from fossil fuels, increases. Peanuts (Arachis hypogaea L.), when infected by an effective Rhizobium strain, form nodules which can fix amounts of N_2 adequate to produce normal plant growth in soils that have rel - tively low levels of available N. If the peanut could fix N_2 mor efficiently, higher yields might be possible and more residual N could remain in the soil for the succeeding crop.

One method of improving the N_2-fixing ability of peanut woul be to develop cultivars that can fix N_2 more efficiently. To mak efficient genetic gains in the N_2-fixing ability, a knowledge of h ow this characteristic is inherited is required. One heritable trai which is a primary component of N_2-fixing ability is nodulation. One objective of this study was to investigate the inheritance of nodulation in peanut.

The peanut lines that were used as parents in this study had different testa colors. Thus, another objective of this study wa to determine if any of the genes controlling testa color were lin ed to the gene or genes that control nodulation.

CHAPTER 2
LITERATURE REVIEW

Inoculating Peanuts with Rhizobium

There have been many studies to determine the effects of inoculating peanuts (Arachis hypogaea L.) with Rhizobium. Duggar (19) found that peanut yield was increased 30 to 40% when unshelled Spanish peanuts were inoculated and planted. Also, a significant positive correlation was found between the number of nodules per plant and yield of dry peanuts per plant.

Schiffmann (53) reported that peanut yield, 1000 pod weight, 1000 seed weight, and crude protein content of hay were all higher in plots that were inoculated with effective Rhizobium strains.

Van Der Merwe et al. (64) and Walker et al. (67) found no significant increase of seed yield due to inoculation with Rhizobium. Iswaran and Sen (32) reported that a recommended rate of inoculant resulted in no yield increase but a 10x inoculation rate induced a yield response.

Ratner et al. (50) found that pod yield of inoculated plots was significantly higher than of uninoculated plots. Schiffmann and Alper (55) reported that best results were obtained when inoculant was placed in the seed furrow, and that as nodule number per plant increased the average nodule dry weight decreased. They (54) also evaluated the effect of placing the inoculant at a depth of 8, 12, or 15 cm. As the placement depth increased, the yield, 1000 seed weight, nodule number per plant, and nodule dry weight per plant

decreased. They also observed that when there were fewer nodules per plant, there was an increase in average weight per nodule.

According to Tonn and Weaver (63), the Virginia type cultivars 'Florunner' and 'Florigiant' had more N in their vegetative organs, accumulated N in their pods at a faster rate, developed more nodule mass, and had a higher C_2H_2 reduction rate than the two Spanish cultivars, 'Starr' and 'Tammut 74.' Duggar (20) found that Spanish peanuts averaged 11 nodules per plant at harvest and runner peanuts averaged 127 nodules per plant.

Nambiar et al. (38) randomly selected six lines from each of the commercially grown botanical varieties and found that plants of A. hypogaea subsp. hypogaea var hypogaea had more nodules on the hypocotyl than the other two botanical varieties. They stated nodule formation on the hypocotyl may be a desirable trait because it has been observed that nodules on the hypocotyl remain active longer than those on the root.

Peanut-Rhizobium Interaction

The symbiotic relationship of peanut and Rhizobium has been investigated by many researchers. Whiting and Hansen (68) found that peanut is a member of the cowpea cross-inoculation group.

Allen and Allen (2) inoculated two peanut cultivars with 59 strains of Rhizobium that were isolated from 31 different leguminous species and found that all were infective. Gaur et al. (22) observed that Rhizobium obtained from 51 legume species representing 23 genera nodulated peanut plants. They (21) also reported that peanut

nodulated well in desert soil where no Arachis species had grown previously. This provided evidence that the peanut is less specific in its Rhizobium requirement than most legumes.

In peanut inoculation studies, Chandler (16) found that in contrast to Trifolium spp., no infection threads were formed. Root hairs were found only where there was an emerging lateral root and only root hairs with large basal cells were infected. Similar to Trifolium spp., root hairs were deformed when rhizobia were present but the rhizobia entered at the junction of root hairs and the epidermal and cortical cells. The bacteria moved intercellularly in the middle lamellae and entered the cortical, root hair, and large basal cells through the structurally altered cell wall. These invaded host cells divided repeatedly to form the nodule tissue, and when the host cells stopped dividing, bacteroids were formed.

Bhuvaneswari et al. (10) also reported peanut had nodules only where lateral roots emerged. No nodules were observed at the base of laterals where well-developed root hairs were present at the time of inoculation. Nodules developed at 25 to 50% of the sites where lateral roots emerged at the time of inoculation but basal root hairs emerged after inoculation. Nodules developed at 80 to 100% of the laterals which emerged after inoculation. Susceptibility of the peanut root to infection by Rhizobium may be more related to root hair development than to lateral root emergence (10).

Allen and Allen (2) observed spherical, plastid-like bodies in the bacteroidal area of infected cells. Staphorst and Strijdom (59) found the spherical structures located in mature nodules on peanut

roots were Rhizobium bacteroids. The nodules from peanut were the only ones that contained spheroplast-like cells and 13 other species contained "typical" bacteroid and rod-shaped cells. This characteristic of spheroplast-like cells in nodules seems to be a property of the genus Arachis because Staphorst and Strijdom (59) also observed the spheroplast-like cells in A. erecta, A. nambyquarae, A. villosulicarpa and an unidentified Arachis spp. Van Rensberg et al. (65), using electron microscopy, reported the spheroplast-like cells were protoplasts devoid of cell wall material.

Sen and Weaver (56) found that peanuts had three times more N in their plant tops per unit weight of nodules than cowpeas (Vigna unguiculata L. Walp) when inoculated with the same strain of Rhizobium.

Inheritance of Non-Nodulation and Ineffective Nodulation

There have been several reports on the inheritance of non-nodulation for species which normally nodulate. Nutman (42) found non-nodulation in red clover (Trifolium pratense L.) to be controlled by a recessive gene (r) and affected by a maternally transmitted component. Two additional factors proposed as influencing the inheritance of nodulation were dilution of a cytoplasmic factor and the presence of zygotic and post-zygotic lethals.

Williams and Lynch (69) crossed a non-nodulating soybean (Glycine max L. Merr.) with a nodulating line and then classified the roots of F_1, F_2, F_3, and F_1BC_1 plants as nodulated or non-nodulated. They determined that nodulation was controlled by one dominant gene with non-nodulating plants being homozygous recessive.

Gorbet and Burton (24) described a non-nodulating peanut line which was originally identified in the F_3 generation from the hybridization of UF 487A, a University of Florida breeding line, with PI 262090. They concluded that non-nodulation was not controlled by a single recessive gene.

Nigam et al. (39) determined the genetics of a non-nodulating peanut which was identified from the crosses of PI 259747, with two Virginia cultivars, 'NC 17' and 'NC Ac 2731.' They found that two independent genes controlled nodulation with the non-nodulating plants being homozygous recessive at both loci.

According to Holl (30), a mutant line of Pisum had one gene $\underline{Sym}_2$ controlling nodulation and another gene $\underline{Sym}_3$ controlling N_2 fixation when nodules were present. The two genes segregated independently with nodulation and N_2 fixation being dominant to non-nodulation and no N_2 fixation.

Peterson and Barnes (48) found three alfalfa (Medicago sativa L.) clones in which ineffective nodulation was controlled by a single tetrasomically-inherited recessive gene symbolized as $\underline{in}_1$, $\underline{in}_2$, or $\underline{in}_3$. With a fourth clone they found that ineffective nodulation was controlled by two recessive genes symbolized as $\underline{in}_4$ and $\underline{in}_5$. Ineffective nodules were produced when both loci were nulliplex. The non-nodulating trait was controlled by two recessive genes, symbolized $\underline{nn}_1$ and $\underline{nn}_2$ with non-nodulating plants being nulliplex at both loci. Data for all F_2 and backcross families with two of the clones showed consistent deficiencies of about 28% ineffective plants and expected ratios were calculated assuming a 28% deficiency

of the nulliplex genotype. A similar adjustment was made for another clone that showed consistent deficiencies of 32% ineffective plants.

Vest and Caldwell (66) found that the soybean cultivar 'Hill' was ineffectively nodulated by Rhizobium japonicum (Kirchner) strain 61. This trait was controlled by a single gene and ineffective nodulation was dominant. Some plants had a few normal-appearing nodules and produced progeny that either segregated or were all ineffectively nodulated. Thus, these plants with a few normal-appearing nodules were considered ineffectively nodulated.

Caldwell et al. (14) reported that the soybean cultivar 'Merrill' has an ineffective nodulation response to the R. japonicum serogroups 3-24-44 and 122. When Merrill was inoculated with strains of the serogroup 3-24-44, many small white (tumor-like) nodules but no normal nodules were formed. When Merrill was inoculated with strains of serogroup 122, a few normal-size nodules and a very few small white nodules developed. Caldwell (13) also reported that a single dominant gene Rj_2 caused ineffective nodulation of soybeans by certain strains of serogroups 3-24-44 and 122 of R. japonicum.

Nutman (43) identified two red clover clones which were ineffectively nodulated by Rhizobium trifolii Dang. strain A. When he investigated the inheritance of this trait, he scored plants from 0 to 4 with 0 being completely ineffective and 4 being normally effective. When he analyzed the results of segregating generations, he considered half of the plants scored 1 as effective and half ineffective. With this adjustment, Nutman (44) concluded that the ineffective response

to strain A was controlled by a recessive allele at one locus but that it was also modified by other recessive characters.

Gibson (23) found that the 'Northern First Early' variety of Trifolium subterraneum L. formed ineffective nodules with the normal effective NA30 strain of R. trifolii. When Northern First Early was crossed with other varieties of T. subterraneum, the F_1 plants were intermediately effective. In the F_2 generation plants were scored as ineffective, intermediate, and effective for nodulation. While the F_2 data did not fit a 1:2:1 ratio, Gibson (23) concluded that a single locus with major effects and modifying genes probably controlled the plants' response to strain NA30.

Penetrance and Expressivity

Penetrance and expressivity have each been defined (1) as the frequency with which a gene produces a recognizable effect, and the degree or amount that a genetic character affects the phenotype, respectively. The penetrance and expressivity of a genetic character can be altered by genetic background.

Loesch (35) investigated five x-ray-induced morphological mutants from the peanut cultivar 'NC4.' He concluded that the variable expressivity of the mutant phenotypes observed in the F_2 and F_3 generations was caused by differences in the background genotype.

Gottschalk (25) transferred the bif-1 gene, which caused bifurcated main stems, into the genomes of other Pisum mutants. The gene efr, which caused early flowering, reduced the penetrance of bif-1. When efr and ion were combined there was no further reduction in the

penetrance of bif-1. A gene (sg-1) which caused a reduction in grain size increased the penetrance of bif-1.

There have been several reports in which adjustments were made to the data or the expected values when investigating the inheritance of a trait with incomplete penetrance. These include four reports described previously in this chapter (44, 48, 66). Harris et al. (27) working with corn (Zea mays L.), investigated the inheritance of second ear shoots that silk (SES). Lines that did not have 100% SES were designated AA, while lines that did have 100% were designated aa. To calculate the expected frequencies of SES in the F_2 and F_1BC_1 generations, the degree of penetrance of the parental genotypes were used. For example, from the cross AA x Aa, the segregation would be 1/2 AA:1/2 Aa. The expected frequency of SES would be 1/2 AA SES (from the parental data) + 1/2 Aa SES (from the F_1 data). All goodness-of-fit and heterogeneity chi-square values were nonsignificant when the observed and expected values were compared. They concluded that the SES trait was controlled at one locus with the aa genotype having 100% SES.

Sorells et al. (58) investigated the inheritance of second ear formation in corn, a trait with incomplete penetrance. They used a technique for analyzing their data that was similar to that used by Harris et al. (27).

The spotted leaf trait in alfalfa was reported by Azizi and Barnes (9) to be controlled by two tetrasomic genes, SA and SB, with random chromosome inheritance. The genotypes SA- - - and sasasasa sbsbsbsb prevented leaf spotting, whereas the genotype sasasasa

SBSB- - produced spotted leaves. If the simplex SB genotype sasasasa SBsbsbsbsb caused spotted leaves or normal leaves, then the S_1 progeny would segregate 3 spotted:1 normal or 1 spotted:3 normal, respectively. However, the S_1 progeny of a SB simplex genotype segregated 1 spotted: 2 normal. All expected ratios were adjusted so that 20% of the plants with simplex SB genotypes were expected to produce the spotted leaf trait. When this adjustment was made, the segregation observed from 13 different crosses supported the original genetic hypothesis.

Working with barley (Hordeum vulgare L.), Carroll et al. (15) investigated the inheritance of resistance to seed transmission of barley stripe mosaic virus (BSMV). 'Vantage,' the susceptible variety, had a relatively high rate of seed transmission of BSMV, ranging from 66.3 to 80.9%. Because of incomplete penetrance the classification of F_2 plants as being resistant was not reliable. When F_2 plants were progeny tested to determine the F_2 genotypes, the F_2 data then fit a 1:3 ratio for resistant and susceptible, respectively. Thus, resistance was being controlled by a recessive gene.

Paternal Inheritance

Working with corn, Lin (34) found that there was a 50% reduction of kernel size with the hypoploid-endosperm class when B translocations had a breakpoint near du in 10L. B translocations with a breakpoint further from du have only a 5% reduction of kernel size. Lin (34) found that with the TB-10 [19] translocation, kernels of the 2♀:2♂ endosperm class were normal in size like the normal endosperm (2♀:1♂) class. Kernels of the 4♀:0♂ class had a 50% reduction in seed size,

like the hypoploid endosperm (2♀:0♂) class. The two examples with tetraploid endosperms had different phenotypes. Thus, Lin (34) concluded that a paternal form of the chromosome region investigated is needed for normal endosperm development.

Crouse (18) reported that cells of a developing Sciara embryo can differentiate between maternally and paternally derived homologous chromosomes and between the sex chromosome (X) and the autosomes. Evidence of this ability is found in the unusual cytogenetic behavior found in several species of Sciara. During the first spermatocyte division there is selective elimination of the paternal homologues. During the second division the maternally derived X chromosome does not divide; thus, the sperm nucleus contains two identical X's and three autosomes. Gamete formation by the female is through normal meiosis. The zygote then contains an extra X chromosome; however, during embryo development, one of the paternally derived chromosomes is eliminated from the somatic nucleus of the females and from the germ cells of both sexes. Both paternally derived X chromosomes are eliminated from the somatic nuclei of males. Crouse reported that "the dramatic chromosome unorthodoxies in Sciara are clearly unrelated to the genic make-up of the chromosomes: a chromosome which passes through the male germ line acquires an 'imprint' which will result in behavior exactly opposite to the 'imprint' conferred on the same chromosome by the female germ line." (18, P. 1442) In other words, the imprint a chromosome bears is unrelated to the genic constitution of the chromosome and is determined only by the sex of the germ line through which the chromosome has been inherited.

Simon and Peloquin (57) investigated the inheritance of the origin of callus growth (anther or filament) during anther culture of Solanum hybrids. Stamens from five to twenty plants of each species or hybrid were cultured. Callus growth for each stamen was categorized as originating from the filament (F) or anther (A). A characteristic of each species and hybrid was that callus formation originated predominately from the F or the A. When a hybrid was made by crossing an A species (female) with an F species (male) the hybrid was F. When the reciprocal cross was made the hybrid was A. Simon and Peloquin (57) believed this type of inheritance could be caused by exclusive male transmission of a cytoplasmic factor. This was supported by the research of Nilsson-Tillgren and von Wettstein-Knowles (40) who demonstrated that the male plastome was still present in uninucleate pollen. Also, Kutzelnigg and Stubbe (33) have shown that for some plastome mutants in Oenothera a cytoplasmic factor was transmitted only through the pollen. Further evidence to support this possibility was obtained when Tilney-Bassett (62) carefully analyzed normal and mutant plastids of Pelargonium zonale L. and their effects on fertilization and stages of embryo survival. They concluded that plastid transmission can be predominately paternal in this species. The second explanation for this type of inheritance was that paternal genes, possibly those near the locus controlling andric expression, were imprinted.

Simon and Peloquin (57) proposed that by making paired backcrosses one could determine if this unusual mode of inheritance was caused by male transmission of a cytoplasmic factor (paternal

inheritance) or by imprinting of paternal genes. From the crosses A x (A x F) and F x (F x A) paternal inheritance would produce all F or all A plants, respectively, while imprinting would produce equal numbers of A and F plants in both crosses. The crosses (A x F) x A and (F x A) x F would produce all A plants or all F plants, respectively, for both paternal inheritance or imprinting.

Mouli and Patil (37) investigated the inheritance of foliaceous stipule in the peanut. F_1 plants had normal stipules when a normal line was the male parent, but when a normal line was used as the female parent, the F_1 had foliaceous stipules. The segregation in the F_2 was similar in reciprocal crosses. Three of the crosses segregated 12:4 and one segregated 1:1, normal:foliaceous, respectively, in the F_2. To confirm the reciprocal differences found in the F_1 generation the following crosses were made with the resulting F_1BC_1 phenotypes:

Foliaceous F_1 x normalσ - 28 normal plants

Normal x foliaceous $F_1\sigma$ - 14 normal:8 foliaceous

Normal F_1 x normalσ - 20 normal plants

Normal x normal $F_1\sigma$ - 14 normal:6 foliaceous

The results in both the F_1 and F_1BC_1 generations provided strong evidence that the expression of the foliaceous stipule phenotype was dependent on the male gamete. A dihybrid model was proposed on the assumption that the foliaceous stipule was expressed only when the pollen contained both recessive genes. The normal parent that produced a 1:1 segregation in the F_2 was homozygous recessive at one of

the loci, but the other three normal parents that produced 12 normal: 4 foliaceous segregation in the F_2 were homozygous dominant at both loci. Results from the F_3 generation also confirmed this proposed mode of inheritance.

Cytoplasmic Inheritance in Peanut

There have been a few studies that have indicated that different peanut cytoplasms may influence the inheritance of some characters.

Ashri (3) made reciprocal crosses between 'Virginia Beit Dagan No. 4' (V4) and six other peanut varieties which all have a bunch growth habit. In all crosses the F_1's were bunch when V4 was the female parent. When V4 was the male parent, the F_1's had a runner growth habit. The reciprocal crosses were evaluated for growth habit in the F_2, F_3, F_1BC_1, and F_2BC_1 generations and it was concluded that there were two plasmon types. One plasmon type (V4) was only found in V4 and the other plasmon ("others") was present in the other six varieties. To explain the inheritance of growth habit it was proposed that the two genes $\underline{Hb_1}$ and $\underline{Hb_2}$ interact differently with each plasmon. Ashri (4) further reported that when in the V4 plasmon the $\underline{Hb_1}$-$\underline{Hb_2}$- genotype was runner-type while all other genotypes had bunch growth habit. In the "others" plasmon at least three dominant alleles were required to produce a runner, and plants with two or less dominant alleles produced plants with a bunch growth habit.

Resslar and Emery (51), using two of Ashri's (3, 4) cultivars, proposed that the reciprocal differences observed in the F_1 and F_2 generations were caused by dissipating maternal effects and not cytoplasmic inheritance.

In another study, Ashri (8) found that the HG1 cultivar had a third plasmon (G) and a third locus ($\underline{Hb_5}$) which affect growth habit.

Coffelt and Hammons (17) determined the inheritance of pod constriction in peanuts. No differences were detected in F_1's when reciprocal crosses were made between 'Argentine' (unconstricted) and 'Early Runner' (constricted). However, reciprocal differences were found in the F_2. They proposed that three unlinked nuclear loci and one cytoplasmic factor controlled pod constriction in this cross. When any two of the four factors were homozygous recessive, the plant produced unconstricted pods.

Patil and Mouli (47) crossed a dwarf peanut which originated as a spontaneous mutant from the peanut cultivar 'Kupergaon-3' with six other cultivars. Reciprocal differences were observed in the F_1's for plant height and secondary branching and they were assumed to be caused by the interaction of nuclear and cytoplasmic factors.

Parker et al. (45) used six peanut cultivars in a diallel cross. The F_1 plants were evaluated for 17 seedling characters. Maternal effects were significant for leaf width at 18 days. Maternal and reciprocal effects were significant for number of leaves on cotyledonary branches at 15 days.

Isleib et al. (31) assessed the quantitative genetic aspects for N-fixing ability with a diverse group of peanut cultivars. The following characters were measured: nitrogenase activity, number of nodules, shoot dry weight, N content of the shoot, and dry weight per nodule. Reciprocal effects were observed for nodule number, nitrogenase activity, and total N. Interaction between nuclear and extranuclear

factors are generally believed to cause these effects. Maternal effects were significant for all the traits except nitrogenase activity. Maternal effects are generally thought to be caused by heritable extranuclear factors, such as DNA in mitochondria and chloroplasts.

Inheritance of Testa Color in Peanut

There have been many reports on the genetics of testa color in peanut. A thorough review of this topic has been presented by Hammons (26).

Stokes and Hull (60) found red testa dominant to tan and controlled at one locus. Hayes(29) crossed 'Valencia' with 'Sine.' They had dark red and pale brown testa, respectively. He found that testa color was controlled at one locus with red being dominant.

Prasad and Srivastava (49) found that purple was dominant to rose and was controlled at two loci by duplicate genes. They also found that rose was dominant to light rose and was controlled by duplicate genes at two loci. In a cross between purple and light rose, the F_1 was purple and the F_2 data fit a 255 purple:1 light rose ratio. They concluded there was a tetragenic difference between purple and light rose with purple being dominant.

Ashri (5, 7) provided evidence that two loci controlled red testa color. At the $\underline{R_1}$ locus, the dominant allele $\underline{R_1}$ gives red color, but at the $\underline{R_2}$ locus the recessive $\underline{r_2}$ allele gives the red color.

Harvey (28) showed that red was dominant to pink and was controlled at one locus in the germplasm he was using. He also found

that purple was incompletely and monogenically dominant to pink and that the dominant gene for red testa affected the degree of purple pigmentation. Stokes and Hull (60) reported that the variegated testa of A. nambyquarae was incompletely dominant to the solid color of A. hypogaea testa.

Branch and Hammons (11, 12) found that inheritance of red on white testa variegation in peanut fit a genetic model for incomplete dominance at one locus. The genotypes designated VV, Vv, and vv produced the phenotypes variegated, trace amount of variegation, and no variegation, respectively.

The inheritance of inner testa color in peanuts was reported to be controlled by at least four loci by Rodriguez and Norden (52). They found that a dominant allele (S) caused the inner testa color to be a neutral white.

CHAPTER 3
INHERITANCE OF NON-NODULATION IN PEANUT

Introduction

The peanut (Arachis hypogaea L.) is a legume which, when infected by effective Rhizobium strains, will form nodules on the root which are capable of N_2 fixation. This characteristic is common to all legumes except those belonging to the subfamilies Caesalpinioideae and Mimosoidaea.

Reports in five species that normally nodulate indicate the presence of a gene or genes which cause a plant to be non-nodulated. A single recessive gene caused non-nodulated plants in soybeans (Glycine max L. Merr.) (69) and peas (Pisum spp.) (30). Nutman (42) reported non-nodulation in red clover (Trifolium pratense L.) to be controlled by a recessive gene (r) and affected by a maternally transmitted component. He also proposed that dilution of the cytoplasmic factor and zygotic and post-zygotic lethals influenced the inheritance of nodulation. Non-nodulation in alfalfa (Medicago sativa L.) was reported to be caused by two tetrasomically inherited recessive genes (48).

Gorbet and Burton (24) described a non-nodulating peanut which was originally identified in the F_3 generation from the hybridization of UF 487A, a University of Florida breeding line, with PI 262090. Nigam et al. (39) also identified non-nodulating peanut plants from the cross of PI 259747 with 'NC 17' and 'NC Ac 2731.' They reported

that two independent duplicate genes control nodulation and that non-nodulated plants are homozygous recessive at both loci.

The objective of this study was to investigate the inheritance of nodulation in peanut using a non-nodulating peanut line, M4-2, selected from the non-nodulating germplasm described by Gorbet and Burton (24).

Materials and Methods

Four peanut genotypes (Arachis hypogaea subsp. hypogaea var hypogaea) were used as parents in this study and are described in Table 3-1. A diallel cross was made with the four parents and selected F_1 plants were backcrossed to M4-2 or PI 262090. All crosses were made in the greenhouse using the method described by Norden and Rodriguez (41). All subsequent generations were field grown at the University of Florida Agricultural Research Center at Marianna, Florida, during the four growing seasons 1979-82 (Table 3-2). Recommended agronomic practices were utilized including inoculation of seed at planting with cowpea-type Rhizobium sp. manufactured by Nitragin®.[1]

Leaf color ratings of individual plants were taken in the field by pulling a representative leaf from each plant and matching it to a color on "The Munsell Limit Color Cascade." These ratings were taken just prior to digging on individual F_1 and F_1BC_1 plants in 1981 and 1982 and on F_2 plants in 1980 and 1981. Individual plants

[1]The listing of specific trade names does not constitute endorsement of these products by the Florida Agricultural Experiment Station in preference to others containing the same components.

Table 3-1. A description of the peanut lines used as parents in crosses made to investigate the inheritance of non-nodulation.

Parent	Nodule classification	Description or source
M4-2	Non-nodulating	A line selected from the cross UF 487A x PI 262090
PI 262090	Normal	Plant harvested from farm near Roboré, Bolivia
UF 487A	Normal	University of Florida breeding line
Florunner	Normal	Cultivar

Table 3-2. The generations of peanuts grown each year with a description of the plot size, number of seed planted per plot, rows per plot, seed spacing, and age when dug.

Generation	Year	Plots per entry	Seed planted per plot	Rows per plot	Within row seed spacing	Plant age at digging
			no.		cm	days
F_1	1979	1	2-8	1	57	120
F_1	1981	1	2-8	1	57	127
F_1	1982	1	2-8	1	57	120
F_1BC_1	1981	1	2-8	1	57	127
F_1BC_1	1982	1	2-8	1	57	120
F_2	1980	5	32	2	38	103-145†
F_2	1981	5	32	2	38	106-126
F_2	1982	3	60	2	19	93- 98
F_2BC_1	1982	1	60	2	19	85- 98
F_3	1981	1	60	2	19	101-146
F_3	1982	1	60	2	19	75- 99

†Indicates the range of number of days from planting to digging.

were tagged so that foliage color could be compared with nodule characteristics for each plant. Plants were dug using a conventional peanut digger-inverter with the cutting blades set as deep in the soil as possible. Most roots were cut at about 20-25 cm below the soil surface. Nodulation of roots of individual plants was rated as described in Table 3-3 immediately after digging. Pods were hand-picked from individual plants that were to be progeny tested. Data were analyzed by chi-square tests for goodness-of-fit to the proposed model.

Results and Discussion

Leaf colors were yellow-green or dark-green and only a few plants had a color between these two extremes. Using the Munsell notation, the typical yellow-green plant was 5.0 GY 4.5/8.2, and the typical dark-green plant was 8.2 GY 3.2/6.1. Yellow-green plants had nodule ratings of 0, 1, or 2 and dark-green plants had nodule ratings of 3, 4, or 5. Plants that had an intermediate leaf color were rated 0, 1, or 2 for nodulation and had less plant competition near them, e.g. a plant at the end of a plot. These plants probably had darker green foliage than within plot plants with nodule ratings of 0, 1, or 2 because they could utilize a larger soil area to extract N. Some plants with nodule ratings of 3, 4, or 5 had an intermediate foliage color, which was apparently induced by stress conditions due to infection by Sclerotium rolfsii Sacc. or attack by lesser cornstalk borer (Elasmopalpus lignosellus Zeller).

Table 3-3. Description of nodulation ratings used to classify individual plants in all field plots.

Nodule rating	Nodule classification	Description of phenotype
0	Non-nod	No nodules
1	Few	1-10 large† nodules
2	Few	11-50 large† nodules
3	Normal	> 50 nodules but < than on a normal Florunner plant
4	Normal	Similar to a normal Florunner plant
5	Normal	More nodules than a normal Florunner plant

†Nodules have about twice the diameter of nodules on a normal Florunner plant.

Although there were six different nodule ratings used in this study, there seemed to be only three distinct categories, non-nodulated (rated 0), few nodules (rated 1 or 2), and normally nodulated (rated 3, 4, or 5). Field observations strongly support this classification system. Most plants with few nodules also had larger nodules and a more yellow leaf color than a normally nodulated plant.

The genetic model proposed for the inheritance of nodulation in this study is similar to the model described by Nigam et al. (39), since it involves a pair of independent genes controlling nodulation with the non-nodulating genotype being homozygous recessive at both loci. For this reason, the gene symbols that Nigam et al. (39) proposed, $\underline{N_1}$ and $\underline{N_2}$, are used in this paper.

The genotypes proposed for the parents are given in Table 3-4. The proposed model has the non-nodulating genotype as $\underline{n_1n_1n_2n_2}$ and all other genotypes have normal nodulation except $\underline{n_1n_1N_2n_2}$, which has few nodules when the parental male gamete was $\underline{n_1n_2}$. Evidence to support this is found in Table 3-5. Nearly all the F_1 plants had normal nodulation except those from PI 262090 x M4-2. Most of the F_1 plants from the latter cross had few nodules; but, because this genotype does not have 100% penetrance, some of the plants were non-nodulated. Also, all the F_1 plants that had M4-2 as the pollen source had a higher proportion of plants with a nodulation rating of 3 than the reciprocal crosses. This provides additional evidence that the $\underline{n_1n_2}$ male gamete reduces nodulation. One plant was rated 0 from the cross of UF 487A x M4-2 and one plant rated 1 from the cross Florunner x M4-2. Since the female used in each of these crosses was

Table 3-4. The proposed genotypes for nodulation control of the peanut lines used as parents in crosses that were made to investigate the inheritance of non-nodulation.

Parent	Genotype
M4-2	$\underline{n_1n_1n_2n_2}$
PI 262090	$\underline{n_1n_1N_2N_2}$
UF 487A	$\underline{N_1N_1n_2n_2}$
Florunner	$\underline{N_1N_1N_2N_2}$

Table 3-5. Nodulation ratings of F_1 plants that were field grown in 1979, 1981, and 1982.

Cross ♀	Cross ♂	Nodulation rating† 0	1	2	3	4	Total
		no. of plants					
UF 487A	M4-2	1	0	0	19	13	33
M4-2	UF 487A	0	0	0	0	26	26
PI 262090	M4-2	8	9	15	0	1	33
M4-2	PI 262090	0	0	0	1	29	30
Florunner	M4-2	0	1	0	42	9	52
M4-2	Florunner	0	0	0	4	27	31
UF 487A	PI 262090	0	0	0	0	17	17
PI 262090	UF 487A	0	0	0	0	28	28
Florunner	PI 262090	0	0	0	0	25	25
PI 262090	Florunner	0	0	0	5	13	18
Florunner	UF 487A	0	0	0	3	26	29
UF 487A	Florunner	0	0	0	0	20	20

†0 = no nodules, 1 and 2 = few nodules, 3 and 4 = normal nodulation.

normally nodulated, these could not have been from selfed seed. These were probably examples of plants that have genotypes which should produce a normally nodulated plant; but, because there was not 100% penetrance, plants with few or no nodules were produced. The plant rated 4 from the cross PI 262090 x M4-2 was probably from a selfed seed. Excluding these three exceptions, the F_1 data support the proposed model.

To evaluate the data of segregating generations, it was necessary to adjust the data because of incomplete penetrance. There have been several reports in which adjustments were made to the data or the expected values when investigating the inheritance of a trait with incomplete penetrance (9, 27, 44, 48, 58, 66). Table 3-6 gives the calculated percentage of plants that were rated 0 in the F_2 and produced an F_3 segregating for nodulation, indicating that genetically the plants probably should have had a few nodules. Calculations on the percentage of plants in the F_2 that were rated 1 or 2 whose progeny did not segregate 1:1:2 (non-nod:few:normal), thus indicating that they should have had normal nodulation, are shown in Table 3-7. In Tables 3-6 and 3-7, the data from Florunner x M4-2, UF 487A x PI 262090, and their reciprocals were combined because the genotype of their F_1 plants and their expected F_2 segregation ratios were the same. Also, relatively few F_3 populations from F_2 plants which rated 0, 1, or 2 were available from each cross.

Table 3-8 presents the method used to adjust the F_2 data. All the values used to adjust the data were obtained from Tables 3-6 and 3-7. Also all adjustments are in one direction with a portion of

Table 3-6. Calculation of percentage of non-nodulated F_2 plants that produced an F_3 population segregating for nodulation.

Cross ♀	Cross ♂	F_3 populations (F_2)[0]‡ Total	F_3 populations (F_2)[0]‡ Segregating for nodulation	F_2 plants [0]§
		no.	no.	%
UF 487A	M4-2	127	0	0
PI 262090	M4-2	170	51	30
Florunner x M4-2 & UF 487A	PI 262090	71	22	31

†Also includes reciprocal of cross shown.
‡F_3 populations from an (F_2) rated [0].
§F_2 plants rated [0] that genetically should have had few nodules.

Table 3-7. Calculation of percentage of F_2 plants with few nodules whose progeny did not segregate 1:1:2 (non-nod:few:normal).

Cross†		F_3 populations (F_2)[1]‡			F_3 populations (F_2)[2]¶		
♀	♂	Total	Non-seg. 1:1:2	F_2 plants [1]§	Total	Non-seg. 1:1:2	F_2 plants [2]#
		no.		%	no.		%
UF 487A x M4-2		3	3	100	4	4	100
PI 262090 x M4-2		49	0	0	26	0	0
Florunner x M4-2 & UF 487A x PI 262090		13	1	8	14	8	43

†Also includes reciprocal of cross.
‡F_3 populations from an (F_2) rated [1].
§F_2 plants rates [1] that genetically should have been normally nodulated.
¶F_3 populations from an (F_2) rated [2].
#F_2 plants rated [2] that genetically should have been normally nodulated.

Table 3-8. Method used to adjust the F_2 nodulation data to correct for incomplete penetrance when A, B, C, D, E, and F equal the number of plants rated 0, 1, 2, 3, 4, and 5, respectively.

Cross† ♀	Cross† ♂	Nodulation classification	Adjusted frequency
UF 487A x M4-2		Non-nod	= A
		Normal	= B + C + D + E + F
PI 262090 x M4-2		Non-nod	= A x 0.70
		Few	= (A x 0.30) + B + C
		Normal	= D + E + F
Florunner x M4-2 & PI 262090 x UF 487A		Non-nod	= A x 0.69
		Few	= (A x 0.31)+(B x 0.92)+(C x 0.57)
		Normal	= (B x 0.08)+(C x 0.43)+ D + E + F

†Also includes reciprocal of cross shown.

the plants classified as non-nod being reclassified as few or few being reclassified as normal. For example, the observed F_2 data (Table 3-9) for PI 262090 x M4-2 was 855 non-nod, 313 few, and 1149 normal. Table 3-6 shows that 30% of the F_2 plants in this cross that were rated 0 genetically should have had few nodules, since their progeny segregated in the F_3. To adjust the data, 855 was multiplied by 0.30, which is 257. The adjusted frequency was then obtained by subtracting 257 from 855 and adding 257 to 313. In this cross none of the plants classified as few needed to be reclassified as normal.

Table 3-9 presents the F_2 data with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratios of the proposed genetic model. The cross UF 487A x M4-2 and the reciprocal cross segregated 1:0:3. This indicates that the $\underline{N_1}$ allele is completely dominant to $\underline{n_1}$. The cross PI 262090 x M4-2 segregated 1:1:2 because half of the plants that were heterozygous at the $\underline{N_2}$ locus ($\underline{n_1 n_1 N_2 n_2}$) would have been formed as a result of the union of a $\underline{n_1 N_2}$ female gamete and a $\underline{n_1 n_2}$ male gamete which would produce a plant with few nodules. The crosses Florunner x M4-2, UF 487A x PI 262090, and reciprocals should have produced all normal plants because both parents were homozygous dominant at the $\underline{N_1}$ or $\underline{N_2}$ locus and thus there was no segregation at that locus. The total summed and homogeneity chi-square values for all the F_2 data had probabilities above the 5% level; thus, the F_2 data support the proposed model.

Table 3-9. F_2 data with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the proposed model.

Cross ♀	Cross ♂	F_1 families		Nodule classification: Non-nod	Few	Normal	χ^2 test on adj. freq.: Source	df	χ^2	P
		— no. —		— no. of plants —						
UF 487A x M4-2		12	ER‡	1	0	3				
$N_1N_1n_2n_2$	$n_1n_1n_2n_2$†		O	426	51	1303	Total	12	8.99	
			A	426	0	1354	Summed	1	1.08	0.25-0.50
			E	445	0	1335	Homog.§	11	7.91	0.50-0.75
M4-2 x UF 487A		13	ER	1	0	3				
$n_1n_1n_2n_2$	$N_1N_1n_2n_2$		O	485	54	1386	Total	13	14.97	
			A	485	0	1440	Summed	1	0.03	0.75-0.90
			E	481	0	1440	Homog.	12	14.94	0.10-0.25
PI 262090 x M4-2		18	ER	1	1	2				
$n_1n_1N_2N_2$	$n_1n_1n_2n_2$		O	855	313	1149	Total	36	29.10	
			A	598	570	1149	Summed	2	0.88	0.50-0.75
			E	579	579	1159	Homog.	34	28.22	0.50-0.75
M4-2 x PI 262090		13	ER	1	1	2				
$n_1n_1n_2n_2$	$n_1n_1N_2N_2$		O	668	220	857	Total	26	31.16	
			A	468	420	857	Summed	2	3.10	0.10-0.25
			E	436	436	873	Homog.	24	28.06	0.25-0.50
Florunner x M4-2		15	ER	1	1	14				
$N_1N_1N_2N_2$	$n_1n_1n_2n_2$		O	237	122	1960	Total	30	30.62	
			A	163	155	2001	Summed	2	3.42	0.10-0.25
			E	145	145	2029	Homog.	28	27.21	0.50-0.75

Table 3-9.--continued.

Cross ♀	Cross ♂	F_1 families		Nodule classification: Non-nod	Few	Normal	χ^2 test on adj. freq.: Source	df	χ^2	P
		no.		no. of plants						
M4-2	Florunner	12	ER	1	1	14				
$n_1n_1n_2n_2$	$N_1N_1N_2N_2$		O	198	83	1615	Total	24	11.51	
			A	137	118	1641	Summed	2	2.96	0.10-0.25
			E	119	119	1659	Homog.	22	8.55	<0.995
UF 487A	PI 262090	8	ER	1	1	14				
$N_1N_1n_2n_2$	$n_1n_1N_2N_2$		O	124	57	1037	Total	16	9.12	
			A	86	75	1057	Summed	2	1.25	0.50-0.75
			E	76	76	1066	Homog.	14	7.88	0.75-0.90
PI 262090	UF 487A	7	ER	1	1	14				
$n_1n_1N_2N_2$	$N_1N_1n_2n_2$		O	83	51	892	Total	14	7.25	
			A	57	62	907	Summed	2	0.91	0.50-0.75
			E	64	64	898	Homog.	12	6.34	0.75-0.90
Florunner	PI 252090	6	ER	0	0	1				
$N_1N_1N_2N_2$	$n_1n_1N_2N_2$		O	1	3	910				
PI 262090	Florunner	5	ER	0	0	1				
$n_1n_1N_2N_2$	$N_1N_1n_2n_2$		O	0	1	743				
Florunner	UF 487A	5	ER	0	0	1				
$N_1N_1N_2N_2$	$N_1N_1n_2n_2$		O	0	0	803				
UF 487A	Florunner	3	ER	0	0	1				
$N_1N_1n_2n_2$	$N_1N_1N_2N_2$		O	0	1	458				

†Genotypes of parents.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
§Homog. = Homogeneity.

The number of F_2 families derived from normally nodulated F_2 plants that segregated within each of the proposed ratios are presented in Table 3-10. The expected values were determined by calculating the ratio of each expected F_2 genotype that had normal nodulation and then determining the expected segregation ratio of the progeny for each genotype. For example, the F_2 of M4-2 x PI 262090 would produce normally nodulated plants with the genotypes $\underline{n_1n_1N_2N_2}$ and $\underline{n_1n_1N_2n_2}$ in equal numbers. The $\underline{n_1n_1N_2N_2}$ progeny would not segregate and the progeny of $\underline{n_1n_1N_2n_2}$ would segregate 1:1:2. Thus, the expected values listed in Table 3-10 for the cross M4-2 x PI 262090 are one 0:0:1 and one 1:1:2. The number of families that were in each ratio classification was then tested for goodness-of-fit to the proposed model. All chi-square values had probabilities above the 5% level thus adding additional support to the proposed model.

The F_1BC_1 data are presented in Table 3-11. These data were adjusted for four of the crosses in a manner similar to the method used for the F_2, because of incomplete penetrance. Again, all the chi-square values had probabilities above the 5% level, thus supporting the proposed model. Table 3-12 presents the F_1BC_1 families from normally nodulated F_1BC_1 plants via the same form in which the F_3 data were presented in Table 3-10. These data again support the proposed model with the exception of one population from the cross PI 262090 x (PI 262090 x M4-2) segregating 1:1:2. From this cross all families segregating 1:1:2 should have been progeny from a plant with few nodules. Because the female from the cross was

Table 3-10. The number of F_2 families derived from normally nodulated F_2 plants that segregate within each of the proposed ratios with chi-square test for goodness-of-fit to the proposed model.

Cross ♂	Cross ♀		Ratios (non-nod:few:normal) 0:0:1	1:1:2	1:0:3	1:1:14	χ^2 test df	χ^2	P
			no. of families						
UF 487A x M4-2		ER‡	1 :	0 :	2 :	0			
$N_1N_1n_2n_2$ x	$n_1n_1n_2n_2$†	0	26 :	0 :	61 :	0	1	0.47	0.25-0.50
M4-2 x UF 487A		ER	1 :	0 :	2 :	0			
$n_1n_1n_2n_2$ x	$N_1N_1n_2n_2$	0	47 :	0 :	84 :	0	1	0.37	0.50-0.75
PI 262090 x M4-2		ER	1 :	1 :	0 :	0			
$n_1n_1N_2N_2$ x	$n_1n_1n_2n_2$	0	71 :	60 :	0 :	0	1	0.92	0.25-0.50
M4-2 x PI 262090		ER	1 :	1 :	0 :	0			
$n_1n_1n_2n_2$ x	$n_1n_1N_2N_2$	0	58 :	65 :	0 :	0	1	0.40	0.50-0.75
Florunner x M4-2		ER	7 :	1 :	2 :	4			
$N_1N_1N_2N_2$ x	$n_1n_1n_2n_2$	0	62 :	7 :	16 :	35	3	0.45	0.90-0.95
M4-2 x Florunner		ER	7 :	1 :	2 :	4			
$n_1n_1n_2n_2$ x	$N_1N_1N_2N_2$	0	33 :	3 :	7 :	14	3	1.51	0.50-0.75
UF 487A x PI 262090		ER	7 :	1 :	2 :	4			
$N_1N_1n_2n_2$ x	$n_1n_1N_2N_2$	0	30 :	5 :	7 :	18	3	0.45	0.90-0.95
PI 262090 x UF 487A		ER	7 :	1 :	2 :	4			
$n_1n_1N_2N_2$ x	$N_1N_1n_2n_2$	0	56 :	6 :	13 :	25	3	1.46	0.50-0.75
Florunner x PI 262090		ER	1 :	0 :	0 :	0			
$N_1N_1N_2N_2$ x	$n_1n_1N_2N_2$	0	67 :	0 :	0 :	0	0		

Table 3-10.--continued.

Cross ♀	♂		Ratios (non-nod:few:normal) 0:0:1	1:1:2	1:0:3	1:1:14	χ^2 test df	χ^2	p
			no. of families						
PI 262090 x Florunner		ER	1	0	0	0			
$n_1n_1N_2N_2$ x	$N_1N_1N_2N_2$	O	27	0	0	0	0		
Florunner x UF 487A		ER	1	0	0	0			
$N_1N_1N_2N_2$ x	$N_1N_1n_2n_2$	O	12	0	0	0	0		
UF 487A x Florunner		ER	1	0	0	0			
$N_1N_1n_2n_2$ x	$N_1N_1N_2N_2$	O	10	0	0	0	0		

†Genotypes of the parents.
‡ER = Expected ratio, O = Observed frequency.

Table 3-11. F_1BC_1 data analyzed by chi-square test for goodness-of-fit to the proposed model for inheritance of non-nodulation in peanut.

Entry number	Cross: ♀ x (♀ x ♂) or (♀ x ♂) x ♂		Nodulation† Non-nod	Few	Normal	χ^2 test df	χ^2	P
			—— no. of plants ——					
1	M4-2 x (UF 487A x M4-2) & M4-2 x (M4-2 x UF 487A) $\underline{n_1n_1n_2n_2}$ x $\underline{(N_1n_1n_2n_2)}$†	ER‡	1	0	1			
		O	33	0	38	1	0.35	0.50-0.75
2	(UF 487A x M4-2) x M4-2 & (M4-2 x UF 487A) x M4-2 $\underline{(N_1n_1n_2n_2)}$ x $\underline{n_1n_1n_2n_2}$	ER	1	0	1			
		O	34	1	39			
		A	34	0	40	1	0.49	0.25-0.50
3	M4-2 x (PI 262090 x M4-2) & M4-2 x (M4-2 x PI 262090) $\underline{n_1n_1n_2n_2}$ x $\underline{n_1n_1N_2n_2}$	ER	1	0	1			
		O	12	0	16	1	0.57	0.25-0.50
4	(PI 262090 x M4-2) x M4-2 & (M4-2 x PI 262090) x M4-2 $\underline{n_1n_1N_2n_2}$ x $\underline{n_1n_1n_2n_2}$	ER	1	1	0			
		O	12	3	0			
		A	8	7	0	1	0.22	0.50-0.75
5	M4-2 x (Florunner x M4-2) & M4-2 x (M4-2 x Florunner) $\underline{n_1n_1n_2n_2}$ x $\underline{(N_1n_1N_2n_2)}$	ER	1	0	3			
		O	13	0	40	1	0.01	0.90-0.95
6	(Florunner x M4-2) x M4-2 & (M4-2 x Florunner) x M4-2 $\underline{(N_1n_1N_2n_2)}$ x $\underline{n_1n_1n_2n_2}$	ER	1	1	2			
		O	16	10	26			
		A	11	15	26	2	0.62	0.50-0.75

Table 3-11.--continued.

Entry number	Cross ♀ x (♀ x ♂) or (♀ x ♂) x ♂		Nodulation†			χ^2 test		
			Non-nod	Few	Normal	df	χ^2	P
			—— no. of plants ——					
7	PI 262090 x (M4-2 x PI 262090) &	ER	0	1	1			
	PI 262090 x (PI 262090 x M4-2)	O	3	2	6	1	0.09	0.75-0.90
	$\underline{n_1n_1N_2N_2}$ x $\underline{(n_1n_1N_2n_2)}$	A	0	5	6			
8	(M4-2 x PI 262090) x PI 262090 &	ER	0	0	1			
	(PI 262090 x M4-2) x PI 262090	O	0	0	14	0		
	$\underline{(n_1n_1N_2n_2)}$ x $\underline{n_1n_1N_2N_2}$							

†Genotypes of the parents.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency.

Table 3-12. The number of F_1BC_1 families derived from normally nodulated F_1BC_1 plants that segregate within each of the proposed ratios with chi-square test for goodness-of-fit to the proposed model.

Entry number	Cross: ♀ x (♀ x ♂) or (♀ x ♂) x ♂		Ratios (non-nod:few:normal)† 0:0:1	1:1:2	1:0:3	1:1:14	χ^2 test df	χ^2	P
			no. of families						
1	M4-2 x (UF 487A x M4-2) & M4-2 x (M4-2 x UF 487A)	ER†	0 :	0 :	1 :	0			
		O	0 :	0 :	9 :	0	0		
2	(UF 487A x M4-2) x M4-2 & (M4-2 x UF 487A) x M4-2	ER	0 :	0 :	1 :	0			
		O	0 :	0 :	13 :	0	0		
3	M4-2 x (PI 262090 x M4-2)	ER	0 :	1 :	0 :	0			
		O	0 :	14 :	0 :	0	0		
4	M4-2 x (Florunner x M4-2) & M4-2 x (M4-2 x Florunner)	ER	0 :	1 :	1 :	1			
		O	0 :	13 :	14 :	15	2	0.14	0.90-0.95
5	(Florunner x M4-2) x M4-2 & (M4-2 x Florunner) x M4-2	ER	0 :	0 :	1 :	1			
		O	0 :	0 :	11 :	12	1	0.04	0.75-0.90
6	PI 262090 x (PI 262090 x M4-2)	ER	1 :	0 :	0 :	0			
		O	4 :	1 :	0 :	0	0		
7	(PI 262090 x M4-2) x PI 262090	ER	1 :	1 :	0 :	0			
		O	6 :	4 :	0 :	0	1	0.40	0.50-0.75

†ER = Expected ratio, O = Observed frequency.

PI 262090 and not an F_1 plant, this unexpected segregation ratio could not have been the result of a selfed seed in the F_1BC_1 generation. The plant from which this family originated had a nodule rating of 3, but this was probably an error and the plant should have been rated 2.

The F_1BC_1 families derived from few (1-2) and non-nodulated (0) F_1BC_1 plants are presented in Table 3-13. These data are in agreement with the proposed model. The seven families from non-nodulated F_1BC_1 plants that segregated 1:1:2 should have had few nodules but because of incomplete penetrance of this character, the F_1BC_1 plants were non-nodulated. There were also three families that segregated 1:0:3 from F_1BC_1 plants that were non-nodulated. This was not expected; however, each of these three plants must have been created by an $\underline{n_1n_2}$ pollen grain fertilizing an $\underline{N_1n_2}$ egg. This is another example of the $\underline{n_1n_2}$ male gamete reducing nodulation. The same explanation would also apply to the one family which segregated 1:0:3 from an F_1BC_1 plant with few nodules.

The genetic model that has been proposed in this study to describe the inheritance of nodulation in peanut is similar to the one described by Nigam et al. (39). The similarities are that both models assume that nodulation is controlled by two independent genes and that the genotype of a non-nodulating plant is $\underline{n_1n_1n_2n_2}$. The difference between the two models is that the model described in this study contains a third phenotype classified as plants with few nodules. The model states that the genotype $\underline{n_1n_1N_2n_2}$ produces plants with few nodules when the male gamete is $\underline{n_1n_2}$. If the male gamete is

Table 3-13. The number of F_1BC_1 families derived from few and non-nodulated F_1BC_1 plants that segregated within each of the proposed ratios.

Entry	Cross ♀ x (♀ x ♂) or (♀ x ♂) x ♂		Populations from a non-nodulated F_1BC_1 Ratios (non-nod:few:normal)† 0:0:1		1:1:2		1:0:3	Populations from a F_1BC_1 with few nodules Ratios (non-nod:few:normal) 0:0:1		1:1:2		1:0:3
			no. of families									
1	M4-2 x (UF 487A x M4-2) &	ER†	1	:	0	:	0	0	:	0	:	0
	M4-2 x (M4-2 x UF 487A)	O	11	:	0	:	0	0	:	0	:	0
2	(UF 487A x M4-2) x M4-2 &	ER	1	:	0	:	0	0	:	0	:	0
	(M4-2 x UF 487A) x M4-2	O	17	:	0	:	2	0	:	0	:	0
3	M4-2 x (PI 262090 x M4-2)	ER	1	:	0	:	0	0	:	0	:	0
		O	11	:	0	:	0	0	:	0	:	0
4	(PI 262090 x M4-2) x M4-2	ER	1	:	0	:	0	0	:	1	:	0
		O	8	:	4	:	0	0	:	3	:	0
5	M4-2 x (Florunner x M4-2)	ER	1	:	0	:	0	0	:	0	:	0
	M4-2 x (M4-2 x Florunner)	O	13	:	0	:	0	0	:	0	:	0
6	(Florunner x M4-2) x M4-2	ER	1	:	0	:	0	0	:	1	:	0
	(M4-2 x Florunner) x M4-2	O	13	:	2	:	1	0	:	9	:	1
7	PI 262090 x (PI 262090 x	ER	0	:	0	:	0	0	:	1	:	0
	M4-2)	O	0	:	1	:	0	0	:	2	:	0

†ER = Expected ratio, O = Observed frequency.

$\underline{n_1N_2}$ then the plant will be normally nodulated. Evidence to support this model was provided in every generation but the strongest evidence is provided from the F_1 and F_1BC_1 data. The differences found in F_1 plants of PI 262090 x M4-2 and M4-2 x PI 262090 support the model. These reciprocal differences could have been caused by the interaction of nuclear and cytoplasmic factors or by cytoplasmic factors alone. When cytoplasmic factors are involved, reciprocal differences would be expected also in the F_2 and F_3 generations; however, no reciprocal differences were observed in the F_2 or F_3 in this study (Tables 3-9 and 3-10). The F_1BC_1 data (Table 3-11) also provide strong evidence to support the model. The only crosses that had plants with few nodules (with one exception that was explained earlier) were those that had the potential to produce $\underline{n_1N_2}$ female gametes and $\underline{n_1n_2}$ male gametes. An example of this is found in the comparison of the F_1BC_1 results of Entries 3 and 4 in Table 3-11. The genotypes of the parents used in Entry 3 were $\underline{n_1n_1n_2n_2}$ x $(\underline{n_1n_1N_2n_2})$ so when the male gamete was $\underline{n_1N_2}$ the F_1BC_1 plant produced had normal nodules and a genotype of $\underline{n_1n_1N_2n_2}$. The parental genotypes used in Entry 4 were $(\underline{n_1n_1N_2n_2})$ x $\underline{n_1n_1n_2n_2}$; thus when the female gamete was $\underline{n_1N_2}$, the F_1BC_1 had few nodules and a genotype of $(\underline{n_1n_1N_2n_2})$. The genotype of the plants with normal nodulation from Entry 3 and few nodules from Entry 4 should be the same and data in Table 3-12, Entry 3, and Table 3-13, Entry 4, support this because both segregated 1:1:2.

While the proposed model seems to be strongly supported by data from the F_1, F_2, F_3, F_1BC_1, and F_2BC_1 generations, the model assumes

that the phenotype of two plants will be different even though the genotypes are the same, and that cytoplasm has no effect. Mouli and Patil (37) reported a similar mode of inheritance for foliaceous stipule in peanut. They reported that normal x foliaceous produced foliaceous F_1 plants but that foliaceous x normal produced normal F_1 plants. This is similar to the reciprocal differences found in F_1 plants from PI 262090 x M4-2 and M4-2 x PI 262090. They also reported that all normal plants were produced in the F_1BC_1 generation from the crosses (normal x foliaceous) x normal and (foliaceous x normal) x normal. This is similar to what was found in the F_1BC_1 plants from the crosses (PI 262090 x M4-2) x PI 262090 and (M4-2 x PI 262090) x PI 262090 in which all progeny had normal nodulation. Mouli and Patil (37) also reported that the F_1BC_1 plants segregated for foliaceous and normal stipules from the crosses normal x (normal x foliaceous) and normal x (foliaceous x normal). This is similar to what was found in the F_1BC_1 results obtained from the crosses PI 262090 x (PI 262090 x M4-2) and PI 262090 x (M4-2 x PI 262090), in which the F_1BC_1 generation segregated for plants with few nodules and plants with normal nodualtion. As in this study, Mouli and Patil (37) found no reciprocal differences in the F_2 or F_3. Since these similar findings have both been detected for different characters in the same species, it provides evidence that peanuts may have a mechanism of inheritance for some traits that is quite different from other species. Mouli and Patil concluded that the "modification of the segregation ratio was presumably due to the fact that both the recessive genes had to be present in the pollen carrying the functional factors." (37, P.29) The data in this study also support this type of inheritance.

One possible interpretation for this mode of inheritance was discussed by Crouse (18) working with Sciara and Simon and Peloquin (57) working with Solanum hybrids. They described the inheritance of traits that were controlled by chromosome imprinting. The imprint a chromosome bears is unrelated to the genic constitution of the chromosome and is determined only by the sex of the germ line through which the chromosome has been inherited. The mode of inheritance found at the N_2 locus in this study could then be explained as follows. The imprint a peanut chromosome receives when transmitted through pollen activates the N_2 locus, and the opposite imprint, which causes deactivation of the N_2 locus, occurs when the chromosome is inherited through the egg. This imprint may not alter the N_2 locus but the imprint may affect an element in the N_2 gene control system which could be similar to the gene control system in corn (Zea mays L.) as described by McClintock (36).

The physiological mechanism which causes peanuts to be non-nodulated or have few nodules has not been reported and was not investigated in this study. However, a mutant peanut described by Ashri (6) has characteristics that are similar to peanuts that have few nodules. He observed diminutive plants that developed a normal side branch and called these plants mixed. He reported that when diminutive plants were sprayed with gibberellic acid they started to develop normally. This indicated that mixed plants may be caused by hormone levels which exceed a critical threshold level in certain developing bud primordia. It could be speculated that non-nodulated plants may be the result of plants that are deficient for a hormone. When a plant

has a few nodules the hormone exceeds a critical threshold level in the few locations where nodules are produced.

In summary, a genetic model has been proposed which describes the mode of inheritance of nodulation for the peanut lines used in this study. In the proposed model the non-nodulating genotype is $\underline{n_1n_1n_2n_2}$ and all other genotypes have normal nodulation except $\underline{n_1n_1N_2n_2}$ which has few nodules when the parental male gamete of the plant is $\underline{n_1n_2}$. Further study is needed to determine what induces the alleles at the $\underline{N_2}$ locus to cause different phenotypes as a result of the $\underline{n_2}$ allele being inherited from the maternal or paternal parent.

CHAPTER 4
LINKAGE BETWEEN LOCI THAT CONTROL NODULATION
AND TESTA VARIEGATION IN PEANUT

Introduction

There have been only three reports of linkage in peanut (Arachis hypogaea L.). Patel et al. (46) reported that growth habit and branching type did not segregate independently. They estimated the rate of crossing over between the genes for spreading and branching to be 30%. Patil, as reported by Hammons (26), found that the crossover rate between genes for growth habit and pod reticulation was 40.4% and the crossover rate between genes for stem hairiness and pod reticulation was 31.5%.

Non-nodulating peanuts were first identified by Gorbet and Burton (24) in the F_3 generation derived from the cross of UF 487A, a University of Florida breeding line, with PI 262090. Shortly thereafter, Nigam et al. (39) reported non-nodulating peanuts were identified in the F_2 generation derived from the cross 'NC 17' x PI 259747. They stated nodulation was controlled by two independent genes with the non-nodulating plants being homozygous recessive at both loci.

Branch and Hammons (12) reported that the gene for testa variegation (V_1) was incompletely dominant to solid color, which confirmed an earlier report on the inheritance of testa variegation (11).

In preliminary studies on the non-nodulating peanut, it was found that non-nodulated plants often had variegated testa. In this study, crosses were made in which there would be segregation for both

nodulation and testa variegation. The objective was to determine if the gene controlling testa variegation was linked to a gene(s) controlling nodulation.

Materials and Methods

Four peanut (Arachis hypogaea subsp. hypogaea var hypogaea) genotypes were used as parents (Table 4-1). The crosses, M4-2 x 'Florunner,' M4-2 x UF 487A, PI 262090 x UF 487A, and their reciprocals were made in 1978 and 1980. F_1 plants from M4-2 x Florunner and M4-2 x UF 487A were backcrossed to M4-2 in 1980 and 1981. Crosses were made in a greenhouse using the method described by Norden and Rodriguez (41). Subsequent generations were field grown at the University of Florida Agricultural Research Center, Marianna, Florida, during the four growing seasons of 1979-82. Recommended agronomic practices were utilized including inoculation of seed at planting with cowpea-type Rhizobium sp.

All F_1, F_2, and F_1BC_1 plants were tagged before digging and 30 plants were tagged in selected F_3 plots immediately after digging. Plants were dug using a conventional peanut digger-inverter with the cutting blades set as deep (20-25 cm) in the soil as possible. Nodulation of roots of individual plants were rated as described in Chapter 3 immediately after digging. Pod samples were hand picked from all plants that were tagged. Testa were examined in the laboratory and were classified as solid, trace amount of variegation (trace-v), or variegated, as previously described (11, 12). These data were then analyzed by chi-square tests for goodness-of-fit to the proposed

Table 4-1. A description of the four peanut lines used as parents in crosses to determine if there is linkage between loci that control nodulation and testa variegation.

Parent	Nodulation	Testa color	Genotype	Description or source
M4-2	Non-nodulating	Variegated red-light red	$\underline{VVn_1n_1n_2n_2}$	A line selected from the cross UF 487A x PI 262090
PI 262090	Normal	Variegated red-white	$\underline{VVn_1n_1N_2N_2}$	Plant harvested from farm near Roboré, Bolivia
UF 487A	Normal	Solid pink	$\underline{vvN_1N_1n_2n_2}$	University of Florida breeding line
Florunner	Normal	Solid pink	$\underline{vvN_1N_1N_2N_2}$	Cultivar

model. When chi-square tests were used to analyze F_2 or F_3 data, the data were first adjusted to correct for incomplete penetrance. Reasons for adjusting the data were presented in Chapter 3 and the method used to adjust the data is presented in Table 4-2.

Results and Discussion

All F_1 plants produced seed with trace-v testa and all were normally nodulated with two exceptions (Table 4-3). These results are consistent with the findings of other studies on inheritance of testa variegation (11, 12) and nodulation in peanuts (39, Chapter 3). The testa from F_2 plants segregated into three phenotypic categories, solid, trace-v, and variegated. In some F_2 plants which produced testa with trace-v, the variegated area on the seed was difficult to detect and could not be seen on all the seed. Because some plants that produced seed with trace-v were probably classified as solid, the two categories, trace-v and solid, were combined for analysis of the F_2 data. Total, pooled, and homogeneity chi-square values fit a 3:1 ratio (Table 4-4), thus indicating that testa variegation is controlled at a single locus in these crosses. Based on the allele symbols used in previous studies (11, 12) on inheritance of testa variegation, the solid, trace-v, and variegated phenotypes have the genotypes $\underline{vv}$, $\underline{Vv}$, and $\underline{VV}$, respectively. The F_2 data for nodulation (Table 4-5) have been adjusted as described in Table 4-2 and the total, pooled, and homogeneity chi-square values were not significantly different (Table 4-5) when tested with the genetic model described in Chapter 3. The very low probability values obtained from the pooled

Table 4-2. The method used to adjust the F_2 and F_3 data to correct for incomplete penetrance.

Data Nodule rating:	0	1	2	3	4	5
Testa classification:	S V†	S V	S V	S V	S V	S V
Number of plants:	A B	C D	E F	G H	I J	K L

Adjusted data with 1:0:3 nodulation ratio (non-nod:few:normal)

Nodule classification:	Non-nod		Normal	
Testa classification:	S	V	S	V
Number of plants:	M	N	M	N

M = A

N = B

O = C + E + G + I + K

P = D + F + H + J + L

Adjusted data with 1:1:14 nodulation ratio (non-nod:few:normal)

Nodule classification:	Non-nod		Few		Normal	
Testa classification:	S	V	S	V	S	V
Number of plants:	Q	R	T	U	W	X

Q = A x 0.69

R = B x 0.69

T = (C x 0.92) + (E x 0.57) + (A x 0.31)

U = (D x 0.92) + (F x 0.57) + (B x 0.31)

W = G + I + K + (C x 0.08) + (E x 0.43)

X = H + J + L + (D x 0.08) + (F x 0.43)

†S = Solid + trace-v testa and V = Variegated testa.

Table 4-3. Nodulation ratings of F_1 plants that were field grown in 1979 and 1981.

Cross†		Nodulation rating‡				
♀	♂	0	1	2	3	4
		no. of plants				
UF 487A x M4-2		1	0	0	19	39
UF 487A x PI 262090		0	0	0	0	45
Florunner x M4-2		0	1	0	46	36

†Also includes reciprocal of cross.
‡0 = no nodules, 1 and 2 = few nodules, and 3 and 4 = normal nodulation.

Table 4-4. F_2 variegated testa color data from three crosses with chi-square tests for an expected 3:1 ratio.

Cross† ♀	♂	F_1 families		Testa color‡ S	:	V	χ^2 test Source	df	χ^2	P
		— no. —		no. of plants						
UF 487A x M4-2		10	O§	963	:	361	Total	10	6.88	
			E	993	:	331	Pooled	1	3.65	0.05-0.10
							Homog.¶	9	3.23	0.95-0.98
UF 487A x PI 262090		4	O	239	:	93	Total	4	2.58	
			E	249	:	83	Pooled	1	1.61	0.10-0.25
							Homog.	3	0.97	0.75-0.90
Florunner x M4-2		8	O	737	:	278	Total	8	8.74	
			E	761	:	254	Pooled	1	3.09	0.05-0.10
							Homog.	7	5.65	0.50-0.75

†Also includes reciprocal of cross shown.
‡S = Solid and trace-v, V = Variegated.
§O = Observed frequency, E = Expected frequency.
¶Homog. = Homogeneity.

Table 4-5. F_2 nodulation data with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio.

Cross†				Nodulation classification			χ^2 test on adj. freq.			
♀	♂	F_1 families		Non-nod	Few	Normal	Source	df	χ^2	P
		no.		no. of plants						
UF 487A x M4-2		10	ER‡	1	0	3	Total	10	7.34	
			O	351	4	969	Pooled	1	1.61	0.10-0.25
			A	351	0	973	Homog.§	9	5.73	0.75-0.90
			E	331	0	993				
UF 487A x PI 262090		4	ER	1	1	14	Total	4	5.05	
			O	33	15	284	Pooled	1	0.23	0.50-0.75
			A	23	21	288	Homog.	3	5.22	0.10-0.25
			E	21	21	290				
Florunner x M4-2		8	ER	1	1	14	Total	8	9.99	
			O	98	35	882	Pooled	1	1.01	0.25-0.50
			A	67	57	891	Homog.	7	8.98	0.25-0.50
			E	64	64	888				

†Also includes reciprocal of cross shown.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
§Homog. = Homogeneity.

and the high probabilities for the homogeneity chi-square test indicate that testa variegation and nodulation did not segregate independently (Table 4-6). The F_2 segregation for nodulation from the UF 487A x M4-2 cross was controlled at the $\underline{N_1}$ locus because both parents were $\underline{n_2n_2}$. The evidence for linkage of testa variegation and nodulation in this cross indicates that the $\underline{V}$ and $\underline{N_1}$ loci are linked. Because all plants that were non-nodulated or had few nodules were $\underline{n_1n_1}$, they are grouped into one classification for purposes of analysis and presentation.

The calculated crossover percentages of each population ranged from 6.2 to 20% (Table 4-7). However, within crosses involving each of the three parental combinations UF 487A and M4-2 (Entries 1-5), UF 487A and PI 262090 (Entries 6 and 7), and Florunner and M4-2 (Entries 8-14), the ranges were reduced to 6.2 to 8.0, 10.8 to 11.3, and 9.9 to 20%, respectively. The arcsine transformation of the calculated crossover percentage values were analyzed by grouping them into the three parental combinations and the results (Table 4-8) indicate that there is a significant difference caused by the parental combinations.

Means of the crossover percentage for each parental combination were weighted according to the number of observations in each population. The weighted means were 7.1, 11.2, and 12.2% for UF 487A and M4-2, UF 487A and PI 262090, and Florunner and M4-2, respectively. These values were then used as the best estimates of the crossover percentage for each parental combination when calculating the expected values to be used in chi-square test.

Table 4-6. F_2 segregation for variegated testa color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with no linkage or 50% crossing over.

Cross†	F_1 families		Non-nod and few		Normal		χ^2 test on adj. freq.			
			S	V‡	S	V	Source	df	χ^2	P
	—no.—		—— no. of plants ——							
UF 487A	10	ER§	3	1	9	3	Total	40	1035.36	
x		O	28	311	935	50	Pooled	3	1014.55	0.001
M4-2		A	39	312	924	49	Homog.¶	37	20.81	0.75-0.90
		E	248	83	745	248				
UF 487A	4	ER	6	2	42	14	Total	12	95.95	
x		O	9	39	230	54	Pooled	3	84.20	0.001
PI 262090		A	8	36	231	57	Homog.	9	11.70	0.10-0.25
		E	31	10	218	73				
Florunner	8	ER	6	2	42	14	Total	24	233.73	
x		O	28	105	710	172	Pooled	3	215.68	0.001
M4-2		A	24	101	714	176	Homog.	21	18.05	0.50-0.75
		E	95	32	666	222				

†Results of the reciprocal cross is also included.
‡S = Solid and trace-v, V = Variegated.
§ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
¶Homog. = Homogeneity.

Table 4-7. Calculated crossover percentages with number of observations in each population and a listing of the tables where the data is presented.

Experiment number	Generation	Cross†	Crossover	Number of observations	Table number
			%		
1	F_2	[UF 487A x M4-2]	6.6	1324	4-9
2	F_3	[UF 487A x M4-2]	7.5	1636	4-10
3	F_1BC_1	{M4-2 x [(UF 487A x M4-2)]}	8.0	137	4-11
4	F_2 families	[UF 487A x M4-2]	6.2	346	4-12
5	F_1BC_1 families	{M4-2 x [(UF 487A x M4-2)]}	7.7	52	4-13
6	F_2	[UF 487A x PI 262090]	11.3	332	4-9
7	F_2 families	[UF 487A x PI 262090]	10.8	51	4-12
8	F_2	[Florunner x M4-2]	12.4	1015	4-9
9	F_3‡	[Florunner x M4-2]	12.3	372	4-10
10	F_3§	[Florunner x M4-2]	11.5	476	4-10
11	F_1BC_1	M4-2 x [(Florunner x M4-2)]	14.3	49	4-11
12	F_1BC_1	[(Florunner x M4-2)] x M4-2	20.0	50	4-11
13	F_2 families	[Florunner x M4-2]	9.9	132	4-12
14	F_1BC_1 families	{M4-2 x [Florunner x M4-2]}	12.1	99	4-13

†Cross enclosed in []-{} indicates that the results of the reciprocal cross is also included.
‡Those populations which segregated 1:0:3 for none, few, and normally nodulated plants, respectively.
§Those populations which segregated 1:1:14 for none, few, and normally nodulated plants, respectively.

Table 4-8. Analysis of variance of the arcsine transformation of percentage crossing over calculated on the three parental combinations.

Source	df	MS
Total	13	
Parental combinations	2	47.10**
Error	11	4.05

**Indicates significant difference at 0.01 level.

When the adjusted frequencies for the F_2 generation of each cross were analyzed for goodness-of-fit to the expected frequencies with linkage, no significant differences were found (Table 4-9). The results obtained from F_3 plants which were in F_2 families that were segregating for nodulation and testa variegation are presented in Table 4-10. The F_2 families from the Florunner x M4-2 cross were separated into two groups. The first group segregated 1:0:3, and the second group segregated 1:1:14 (non-nod:few:normal). An insufficient amount of data was obtained from the F_2 families of UF 487A x PI 262090; these data are not presented. There were no significant differences found when these F_2 families were analyzed by chi-square test.

The observed frequencies in the F_1BC_1 generation were not significantly different from the expected frequencies calculated with the indicated crossover percentage (Table 4-11). In Table 4-12, F_2 plants are classified as non-crossover, crossover, or two crossover types by comparing the F_2 plant's testa variegation with the segregation for nodulation in the F_3. For example, if an F_2 population (F_3 plants) segregated 1:1:2 (non-nod:few:normal), the F_2 plant that the family was derived from had the genotype $\underline{n_1}n_1\underline{N_2}n_2$. If there was no crossover when this F_2 plant was produced, then it would also have the genotype $\underline{VV}$ and thus have variegated testa. If one of the gametes that formed the F_2 embryo had a crossover between the $\underline{N_1}$ and $\underline{V}$ loci, then the F_2 plant would have the genotype $\underline{Vv}$ and thus be trace-v. When the numbers of non-crossover, crossover, and two crossover F_2 plants were compared with the expected numbers assuming the appropriate crossover percentage, no significant difference was detected.

Table 4-9. F_2 segregation for variegated testa color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with the appropriate crossover percentage.

Cross†	F_1 families		Non-nod and few S‡	Non-nod and few V	Normal S	Normal V	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants							
UF 487A	10	ER§	0.55	3.45	11.45	0.55	Total	30	31.5	
x		O	28.00	311.00	935.00	50.00	Pooled	3	4.2	0.10-0.25
M4-2		A	39.00	312.00	924.00	49.00	Homog.¶	27	27.3	0.25-0.50
		E	45.00	286.00	948.00	45.00				
		(7.1% CO)#								
UF 487A	4	ER	1.69	6.31	46.31	9.69	Total	12	12.94	
x		O	9.00	39.00	230.00	54.00	Pooled	3	1.65	0.50-0.75
PI 262090		A	8.00	36.00	231.00	57.00	Homog.	9	11.29	0.25-0.50
		E	9.00	33.00	240.00	50.00				
		(11.2% CO)								
Florunner	8	ER	1.83	6.17	46.17	9.83	Total	24	18.99	
x		O	28.00	105.00	710.00	172.00	Pooled	3	4.20	0.10-0.25
M4-2		A	24.00	101.00	714.00	176.00	Homog.	21	14.79	0.75-0.90
		E	29.00	98.00	732.00	156.00				
		(12.2% CO)								

†Results of the reciprocal cross is also included.
‡S = Solid and trace-v, V = Variegated.
§ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
¶Homog. = Homogeneity.
#CO = Crossover.

Table 4-10. F_2 families (F_3 plants) which segregated for variegated testa color and nodulation analyzed by chi-square test for goodness-of-fit to the expected ratio with the appropriate crossover percentage.

Cross†	F_2 families		Non-nod and few S‡	Non-nod and few V	Normal S	Normal V	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants							
UF 487A	56	ER§	0.55	3.45	11.45	0.55	Total	168	201.92	
X		O	53.00	341.00	1174.00	68.00	Pooled	3	5.24	0.10-0.25
M4-2		A	48.00	339.00	1179.00	70.00	Homog.¶	165	196.68	0.05-0.10
		E	56.00	353.00	1171.00	56.00				
		(7.1% CO)#								
Florunner	13	ER	0.92	3.08	11.08	0.92	Total	39	53.41	
x		O	18.00	66.00	260.00	28.00	Pooled	3	5.38	0.10-0.25
M4-2		A	15.00	65.00	263.00	29.00	Homog.	36	48.03	0.05-0.10
		E	21.00	72.00	258.00	21.00				
		(12.2% CO)								
Florunner	16	ER	1.83	6.17	46.17	9.83	Total	48	48.73	
x		O	14.00	57.00	335.00	70.00	Pooled	3	0.81	0.75-0.90
M4-2		A	13.00	50.00	336.00	77.00	Homog.	45	47.02	0.25-0.50
		E	14.00	46.00	343.00	73.00				
		(12.2% CO)								

†Results of the reciprocal cross is also included.
‡S = Solid and trace-v, V = Variegated.
§ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
¶Homog. = Homogeneity.
#CO = Crossover.

Table 4-11. F_1BC_1 segregation for variegated testa color and nodulation with the observed frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with the appropriate crossover percentage.

Cross† ♀ (♀ x ♂) or (♀ x ♂) x ♂		Non-nod and few S‡	:	Non-nod and few V	:	Normal S	:	Normal V	χ^2 test df	χ^2	P
{M4-2	ER§	0.14	:	1.86	:	1.86	:	0.14			
x	O	8.00	:	62.00	:	64.00	:	3.00	3	3.05	0.25-0.50
[(UF 487A x M4-2)]}	E (7.1% CO)¶	4.65	:	63.85	:	63.85	:	4.65			
[(Florunner x M4-2)]	ER	0.24	:	1.76	:	1.76	:	0.24			
x	O	6.00	:	20.00	:	20.00	:	4.00	3	3.49	0.25-0.50
M4-2	E (12.2% CO)	3.10	:	21.90	:	21.90	:	3.10			
M4-2	ER	0.12	:	0.88	:	1.88	:	1.12			
x	O	1.00	:	11.00	:	21.00	:	16.00	3	0.72	0.75-0.90
[(Florunner x M4-2)]	E (12.3% CO)	1.50	:	10.80	:	23.00	:	13.70			

†Cross enclosed in []-{}indicates that the results of the reciprocal cross are also included.
‡S = Solid and trace-v, V = Variegated.
§ER = Expected ratio, O = Observed frequency, E = Expected frequency.
¶CO = Crossover.

Table 4-12. A comparison of testa variegation of F_2 plants with segregation for nodulation in the F_3 and chi-square tests for goodness-of-fit to the expected ratio of parental, crossover, and two crossover types with the appropriate crossover percentage.

Cross†	F_2 testa phenotype‡	F_3 segregation ratios (non-nod:few:normal) 0:0:1	1:1:14	1:0:3	1:1:2	1:0:0		Crossover type§ (NC)	(CO)	(TCO)	χ^2 test df	χ^2	P
		no. of families						no. of families					
UF 487A	V	0 (TCO)	0	12 (CO)	0	112 (NC)	O#	305	41	1			
x	T	8 (CO)	0	127 (NC)	0	12 (CO)	E	299	46	2	2	0.91	0.50-0.75
M4-2	S	66 (NC)	0	9 (CO)	0	1 (TCO)	(7.1% CO)						
UF 487A	V	7 (U)¶	1 (CO)	4 (CO)	11 (NC)	8 (NC)	O	41	9	1			
x	T	12 (U)	12 (NC)	10 (NC)	1 (CO)	2 (CO)	E	40	10	1	2	0.35	0.75-0.90
PI 262090	S	25 (U)	1 (CO)	0 (CO)	0 (TCO)	1 (TCO)	(11.2% CO)						
Florunner	V	15 (U)	7 (CO)	4 (CO)	29 (NC)	20 (NC)	O	108	22	2			
x	T	29 (U)	43 (NC)	16 (NC)	5 (CO)	3 (CO)	E	102	28	2	2	1.77	0.25-0.50
M4-2	S	53 (U)	1 (CO)	2 (CO)	2 (TCO)	0 (TCO)	(12.2% CO)						

†Results of the reciprocal crosses are also included.
‡V = Variegated, T = trace-v, S = Solid.
§(NC) = Non-crossover, (CO) = Crossover, (TCO) = Two crossover.
¶(U) = Cannot classify.
#O = Observed, E = Expected.

Table 4-13 is similar to Table 4-12 except that the F_1BC_1 plants are classified as non-crossover or crossover, instead of F_2 plants. There is no two crossover classification for F_1BC_1 plants because a crossover can be detected only if it occurs in gametogenesis of the F_1 parent. No significant differences were detected when the number of non-crossover and crossover F_1BC_1 plants was compared with the expected values.

These data support the hypothesis that the V and N_1 loci are linked. However, the different crossover percentages observed in the three parental combinations were not expected. One factor that could cause some of the experiments to have a higher crossover rate is incomplete penetrance of normal nodulation. The method used to adjust the data (Table 4-2) assumes independent segregation of the V and N_1 loci, and thus the adjusted values will tend to increase the crossover rate. These adjustments were made in experiments numbered 6, 8, and 10 (Table 4-7). In each of these three experiments the data adjustment has not caused a large increase in the calculated crossover percentage when compared with the crossover percentage found in other experiments of the same parental combination.

If it is assumed the difference in crossover rate observed in the three parental combinations is real and not caused by sampling error, then there are several factors that could affect recombination frequencies in different experiments. Factors known to influence recombination frequencies in Drosophila sp. are sex, maternal age, temperature, cytoplasm, nutrients, radiation, genotype, chromosome-structure, and the position of genes relative to the centromere (61).

Table 4-13. A comparison of testa variegation of F_1BC_1 plants with segregation for nodulation in the F_2BC_1 and chi-square tests for goodness-of-fit to the expected ratio of parental and crossover types with the appropriate crossover percentage.

Cross†	F_1BC_1 testa phenotype‡	F_2BC_1 segregation ratios (non-nod:few:normal)					Crossover type§		χ^2 test		
		1:1:14	1:0:3	1:1:2	1:0:0		(NC)	(CO)	df	χ^2	P
		no. of families									
M4-2	V	0	1 (CO)	0	25 (NC)	0¶	48	4			
x	T	0	23 (NC)	0	3 (CO)	E	48	4	1	0.03	0.75-0.90
(UF 487A x M4-2)						(7.1% CO)					
M4-2	V	2 (CO)	3 (CO)	23 (NC)	22 (NC)	0	87	12			
x	T	20 (NC)	22 (NC)	4 (CO)	3 (CO)	E	87	12	1	0.00	0.995
(Florunner x M4-2)						(12.2% CO)					

†Reciprocal crosses are also included.
‡V = Variegated, T = Trace-v.
§(CO) = Crossover, (NC) = Non-crossover.
¶O = Observed, E = Expected.

Additional studies would be required to prove or disprove that one or more of these factors caused the different crossover rates observed for the different parental combinations. However, the cytoplasm does not seem to be a factor, because no differences were found in the crossover rates of reciprocal crosses. Also, it seems improbable that maternal age would have an effect on crossover rates in peanut. The other factors mentioned may have an effect. For example, gametogenesis occurring at different times during the day in F_1 plants from different crosses could cause a temperature effect on crossover rates. Cytological studies of the parents and F_1 plants could provide evidence of differences in chromosome structure in plants. For example, if UF 487A, Florunner, M4-2, and the F_1 of M4-2 x Florunner all had similar karyotypes, and the F_1 of M4-2 x UF 487A had a similar karyotype to the others except for one chromosome, this would provide evidence, but not proof, that chromosome structure caused the observed differences in crossover rates. It would also provide evidence that the $\underline{N}_1$ and $\underline{V}$ loci are on the chromosome which was different in the F_1 of M4-2 x UF 487A.

Evidence from the F_2, F_3, F_1BC_1, and F_2BC_1 generations have shown that the $\underline{N}_1$ and $\underline{V}$ loci are linked. The recombination frequency between $\underline{N}_1$ and $\underline{V}$ was 7.1, 11.2, and 12.2% for the three parental combinations, UF 487A and M4-2, UF 487A and PI 262090, and Florunner and M4-2, respectively. These data also provide additional support for the genetic model proposed for nodulation in Chapter 3.

CHAPTER 5
A GENE AFFECTING TESTA VARIEGATION COLOR AND ITS ASSOCIATION WITH THE N_2 LOCUS IN PEANUT

Introduction

The inheritance of testa color in peanut (Arachis hypogaea L.) has been the subject of many studies, and an extensive review was presented by Hammons (26). At the R_2 locus the recessive r_2 allele produces red testa color and the dominant R_2 allele produces pink testa (5, 7). The variegated testa of A. nambyqyarae L. was reported as incompletely dominant to solid color of A. hypogaea testa (60). The inheritance of red on white testa variegation in peanut was controlled at one locus and the allele for variegation (V) was incompletely dominant to the allele for solid testa color (v) (11). Recently, Branch and Hammons (12) found that the R_2 and V loci segregated independently with incomplete dominance gene action found at both loci.

Non-nodulating peanuts have been identified in progeny from certain crosses in Florida (24) and India (39). Nigam et al. (39) reported nodulation was controlled by two independent genes with the non-nodulating plants being homozygous recessive at both loci ($n_1n_1n_2n_2$). In Chapter 3, this inheritance was confirmed except that the $n_1n_1N_2n_2$ genotype has few nodules when the male parental gamete was n_1n_2. In Chapter 4 it was reported that the V and N_1 loci are linked.

In this study, crosses were made in which segregation was expected for both nodulation and color of the variegated area (white or light red) of the testa. The objective was to determine the inheritance of color of the variegated area of the testa and to determine if there was any linkage with the N_2 gene that controls nodulation. In addition, crosses were made to determine whether the R_2 locus was linked to the N_1 or N_2 loci. The relationship of the V and R_2 loci was also investigated.

Materials and Methods

Three peanut (Arachis hypogaea subsp. hypogaea var hypogaea) lines were used as parents (Table 5-1). The crosses M4-2 x PI 262090, M4-2 x 'Florunner,' and their reciprocals were made and some of the F_1 plants were backcrossed to M4-2. F_1 plants of M4-2 x PI 262090 were also backcrossed to PI 262090. Crosses were made using the method described by Norden and Rodriguez (41). Subsequent generations were field grown at the University of Florida Agricultural Research Center, Marianna, Florida. Recommended agronomic practices were utilized including inoculation of seed at planting with cowpea-type Rhizobium sp.

All F_1, F_2, and F_1BC_1 plants were tagged before digging and 30 plants were tagged in randomly selected F_3 plots immediately after digging. A conventional peanut digger-inverter was used to dig the plants, cutting the roots 20-25 cm below the soil surface. Nodulation of roots of individual plants were rated as described in Chapter 3 immediately after digging. Pod samples were hand picked from the

Table 5-1. A description of the peanut lines used as parents in crosses made to investigate the inheritance of non-nodulation and testa color.

Parent	Nodulation	Testa color	Genotype	Description or source
M4-2	Non-nodulating	Variegated red-light red (tinted)	$\underline{VVn_1n_1n_2n_2}$	A line selected from the cross UF 487A x PI 262090
PI 262090	Normal	Variegated red-white	$\underline{VVn_1n_1N_2N_2}$	Plant harvested from farm near Robore, Bolivia
Florunner	Normal	Solid pink	$\underline{vvN_1N_1N_2N_2}$	Cultivar

tagged plants and testa were classified as red or pink in the laboratory. When a plant had variegated testa, the lighter colored area of the testa (the variegated area) was classified as white or tinted. When compared with the "Munsell Limit Color Cascade," using Munsell notation, typical pink, red, and tinted testa were 2.4 YR 8.2/4.4, 6.8 R 2.6/9.4, and 8.4 RP 7.3/9.2, respectively.

These data were analyzed by chi-square test for goodness-of-fit to the proposed model. When chi-square tests were used to analyze F_2 data where there was segregation for nodulation, the data were first adjusted to correct for incomplete penetrance. Reasons for adjusting the data were reported in Chapter 3 and the method used to adjust the data is presented in Tables 5-2 and 5-3.

Results and Discussion

All 81 F_1 plants derived from the cross Florunner x M4-2 had pink testa color with a trace amount of variegation. This indicates that pink was dominant to red and that variegation was incompletely dominant to solid testa color. The inheritance of testa variegation from this experiment was presented in Chapter 4 and will not be reported here. The data presented in Table 5-4 indicate that the R_2 allele that produces pink testa color is dominant to the r_2 allele which produces red testa and supports results reported by Ashri (5, 7), but others (29, 60) have reported that red is dominant.

The plants classified as having solid and trace amounts of variegation were grouped together because some plants that produced trace variegated testa were probably classified as having solid (red

Table 5-2. The method used to adjust the data in Table 5-6 to correct for incomplete penetrance.

Data

Nodule rating:	0		1		2		3		4		5	
Testa color:†	P	R	P	R	P	R	P	R	P	R	P	R
No. of plants:	A	B	C	D	E	F	G	H	I	J	K	L

Adjusted data

Nodule classification:	Non-nod		Few		Normal	
Testa color:	P	R	P	R	P	R
No. of plants:	M	N	O	Q	S	T

M = A x 0.69

N = B x 0.69

O = (C x 0.92) + (E x 0.53) + (A x 0.31)

Q = (D x 0.92) + (F x 0.53) + (A x 0.31)

S = G + I + K + (C x 0.08) + (E x 0.47)

T = H + J + L + (D x 0.08) + (F x 0.47)

†P = pink, R = red.

Table 5-3. The method used to adjust the data in Table 5-8 to correct for incomplete penetrance.

Data

Nodule rating:	0		1		2		3		4		5	
Testa variegation:†	W	T	W	T	W	T	W	T	W	T	W	T
No. of plants:	A	B	C	D	E	F	G	H	I	J	K	L

Adjusted data

Nodule classification:	Non-nod		Few		Normal	
Testa variegation:	W	T	W	T	W	T
No. of plants:	M	N	O	Q	S	T

M = A x 0.70

N = B x 0.70

O = (A x 0.30) + C + E

Q = (B x 0.30) + E + F

S = G + I + K

T = H + J + L

†W = white, T = tint.

Table 5-4. Segregation for red and pink testa color with chi-square test for goodness-of-fit to the expected ratio.

Cross	Generation	Families		Testa color			χ^2 test			
				Red		Pink	Source	df	χ^2	P
		no.		no. of plants						
Florunner	F_2	8	ER†	1	:	3	Total	8	4.32	
x			O	262	:	753	Summed	1	0.36	0.50–0.75
M4–2			E	254	:	761	Homog.§	7	3.96	0.75–0.90
Florunner	F_3‡	57	ER	1	:	3	Total	57	66.44	
x			O	496	:	1340	Summed	1	3.97	0.03–0.05
M4–2			E	459	:	1377	Homog.	56	62.47	0.10–0.25
M4–2	F_1BC_1	1	ER	1	:	1	Total	1	0.04	0.75–0.90
x			O	52	:	50				
(Florunner x M4–2)			E	51	:	51				

†ER = Expected ratio, O = Observed frequency, E = Expected frequency.
‡The F_2 families that segregated for testa color.
§Homog. = Homogeneity.

or pink) testa color as reported in Chapter 4. These data (Table 5-5) indicate that in the F_2 generation the two loci, V and R_2, segregate independently and thus support the results of Branch and Hammons (12).

In the F_2 generation of Florunner x M4-2 the expected segregation ratio for nodulation is 1 non-nodulated:1 few nodules:14 normally nodulated. Thus, 3:1:3:1:42:14 is the expected ratio for the segregation of nodulation and testa color (Table 5-6). Because of incomplete penetrance of nodulation, the data in Table 5-6 have been adjusted as described in Table 5-2. These data indicate that the loci N_1, N_2, and R_2 segregate independently.

All of the 66 F_1 plants derived from the cross of PI 262090 x M4-2 had variegated red-white testa color, indicating that white variegation is dominant to tinted. Segregation ratios for the F_2, F_3, and F_1BC_1 generations were not significantly different from the expected ratios, assuming the trait is controlled at one locus with the allele causing white variegation (Wv) being dominant to the allele causing tinted variegation (wv) (Table 5-7). The inheritance of white and tinted variegation color is similar to the inheritance of inner seed-coat color in peanuts reported by Rodriguez and Norden (52). They stated that white inner seed-coat color was dominant. In the F_2 generation derived from the cross PI 262090 x M4-2 the expected segregation ratio is 1 non-nodulated:1 few nodules:2 normally nodulated; thus, 3:1:3:1:6:2 is the expected ratio for the segregation of nodulation and testa variegation color (Table 5-8). These data were adjusted as described in Table 5-3 because of incomplete penetrance and indicate that the N_2 and Wv loci segregate independently.

Table 5-5. F_2 segregation of testa color and variegation analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the two loci $\underline{R}_2$ and $\underline{V}$.

Cross	F_1 families		Pink S†	Pink V	Red S	Red V	χ^2 test Source	df	χ^2	P
	no.		no. of plants							
Florunner	8	ER‡	9	3	3	1	Total	24	27.82	
x		O	554	199	184	78	Pooled	3	4.47	0.10-0.25
M4-2		E	571	190	190	64	Homog.§	21	23.35	0.25-0.50

†S = Solid and trace-v, V = Variegated.
‡ER = Expected ratio, O = Observed frequency, E = Expected frequency.
§Homog. = Homogeneity.

Table 5-6. F_2 segregation of testa color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the three loci, N_1, N_2, and R_2.

Cross	F_1 families		Non-nod Pink	Non-nod Red	Few Pink	Few Red	Normal Pink	Normal Red	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants									
Florunner	8	ER†	3	1	3	1	42	14	Total	40	22.50	
X		O	73	24	27	9	654	228	Pooled	5	1.20	0.90-0.95
M4-2		A	50	17	42	15	662	229	Homog.‡	35	21.30	0.95-0.98
		E	48	16	48	16	666	222				

†ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
‡Homog. = Homogeneity.

Table 5-7. Segregation for white and tinted variegation testa color with chi-square test for goodness-of-fit to the expected ratio.

Cross†	Generation	Families		Variegated testa color			χ^2 test			
				Tinted		White	Source	df	χ^2	P
		no.		—— no. of plants ——						
PI 262090	F_2	11	ER‡	1	:	3	Total	11	10.36	
x			O	336	:	1011	Summed	1	0.00	0.75–0.90
M4-2			E	337	:	1010	Homog.§	10	10.35	0.25–0.50
PI 262090	F_3	40	ER	1	:	3	Total	40	50.62	
x			O	271	:	851	Summed	1	0.43	0.50–0.75
M4-2			E	280	:	842	Homog.	39	50.19	0.10–0.25
M4-2	F_1BC_1	1	ER	1	:	1	Total	1	0.00	0.995
x			O	22	:	22				
(PI 262090 x M4-2)			E	22	:	22				
PI 262090	F_1BC_1	1	ER	0	:	1				
x			O	0	:	22				
(PI 262090 x M4-2)			E	0	:	22				

†Results of the reciprocal crosses are also included.
‡ER = Expected ratio, O = Observed frequency, E = Expected frequency.
§Homog. = Homogeneity.

Table 5-8. F_2 segregation of testa variegation color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the two loci N_2 and Wv.

Cross	Families		Non-nod White	Non-nod Tint	Few White	Few Tint	Normal White	Normal Tint	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants									
PI 262090	11	ER†	3	1	3	1	6	2	Total	55	35.28	
x		O	362	100	157	56	492	180	Pooled	5	4.24	0.50–0.75
M4-2		A	253	70	266	86	492	180	Homog.‡	50	31.04	0.97–0.99
		E	253	84	253	84	505	168				

†ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
‡Homog. = Homogeneity.

In summary, the results of this study support the reports by Ashri (5, 7) that the $\underline{R_2}$ allele, which produces pink testa, is dominant to the $\underline{r_2}$ allele, which produces red testa. They also support the report of Branch and Hammons (12) that the $\underline{R_2}$ and $\underline{V}$ loci segregate independently. It was also determined that the $\underline{N_1}$, $\underline{N_2}$, and $\underline{R_2}$ loci segregate independently. It was shown that testa variegation color is controlled at one locus and that the allele causing white variegation ($\underline{Wv}$) is dominant to the allele controlling tinted variegation ($\underline{wv}$). Finally, it was shown that the $\underline{N_2}$ and $\underline{Wv}$ loci segregate independently.

CHAPTER 6
GENETIC RELATIONSHIP AND INHERITANCE OF NON-NODULATION AND TESTA COLOR IN PEANUT LINES FROM FLORIDA AND ICRISAT

Introduction

The peanut (Arachis hypogaea L.) is a legume which will form root nodules that are capable of N_2-fixation when infected by effective Rhizobium strains. Non-nodulating peanuts have been identified in Florida (24) and India (39). Nigam et al. (39) reported nodulation was controlled by two independent genes, with the non-nodulating plants being homozygous recessive at both loci ($n_1n_1n_2n_2$). Their report was confirmed in Chapter 3 except that the $n_1n_1N_2n_2$ genotype produced plants with only a few nodules when the parental male gamete of the plant was n_1n_2.

The inheritance of testa color in peanut has been the subject of many studies, and an extensive review was presented by Hammons (26). At the R_2 locus the recessive r_2 allele produces red testa color, and the dominant R_2 allele produces pink testa (5, 7). Stokes and Hull (60) reported that the variegated testa of A. nambyquarae L. was incompletely dominant to solid color of A. hypogaea testa. The inheritance of red on white testa variegation in peanut was controlled at one locus, and the allele for variegation (V) was incompletely dominant to the allele for solid testa color (v) (11). Recently, Branch and Hammons (12) found that the R_2 and V loci segregated independently with incomplete dominance gene action found at both loci.

This study was conducted to evaluate the inheritance of nodulation utilizing a non-nodulating peanut line developed from the non-nodulating germplasm described by Gorbet and Burton (24), and two non-nodulating lines developed at ICRISAT. In several of the crosses there was segregation for testa color; these data were also analyzed.

Materials and Methods

Six peanut genotypes were used as parents (Table 6-1). The lines PI 445923 and PI 445924 were crossed with UF 487A, PI 262090, 'Florunner,' and M4-2. Crosses were made using the method described by Norden and Rodriguez (41). About half of the F_1 plants were field grown at the United States Department of Agriculture Research Station at Isabella, Puerto Rico and the remaining F_1 plants were grown in a greenhouse at the University of Florida Agricultural Research Center, Marianna, Florida. The parents and F_2 were grown in the field at Marianna in 1982 using recommended agronomic practices, including inoculation of seed at planting with cowpea-type Rhizobium sp.

All F_2 plants from a sample of the F_1 families were tagged before digging. A conventional peanut digger-inverter was used to dig the plants; roots were cut at 20-25 cm below the soil surface. Nodulation of roots of individual plants was rated as described in Chapter 3. Pod samples were handpicked and testa were classified as pink, red, light purple, or purple and also as solid, trace amount of variegation, or variegated. When compared with the "Munsell Limit Color Cascade," using Munsell notation, typical pink, red, light purple, and purple testa were 2.4 YR 8.2/4.4, 6.8 R 2.6/9.4, 2.6 RP 1.8/5.4, and 4.9 P 2.1/11.2, respectively.

Table 6-1. A description of the peanut lines used as parents in crosses made to investigate the inheritance of nodulation and testa color.

Parent	Nodulation		Test color	Description or source
	Phenotype	Genotype		
M4-2	Non-nodulating	$\underline{n_1n_1n_2n_2}$	Variegated red-light red	A line selected from the cross UF 487A x PI 262090
PI 262090	Normal	$\underline{n_1n_1N_2N_2}$	Variegated red-white	Plant harvested from farm near Robore, Bolivia
UF 487A	Normal	$\underline{N_1N_1n_2n_2}$	Pink	University of Florida breeding line
Florunner	Normal	$\underline{N_1N_1N_2N_2}$	Pink	Cultivar
PI 445923	Non-nodulating	$\underline{n_1n_1n_2n_2}$	Pink	ICRISAT†
PI 445924	Non-nodulating	$\underline{n_1n_1n_2n_2}$	Purple	ICRISAT

†International Crops Research Institute for the Semi-Arid Tropics.

The nodulation data were analyzed by chi-square test for goodness-of-fit to the proposed models for inheritance as described by Nigam et al. (39) and reported in Chapter 3. When the model described in Chapter 3 was used, the data were first adjusted to correct for incomplete penetrance as reported in Chapter 3 and presented in Table 6-2. When there was segregation for nodulation and testa color, the data were adjusted as explained in Table 6-2 with each testa color category adjusted independently.

Results and Discussion

Nodulation

The F_1 data (Table 6-3) for nodulation cannot be fully explained by the genetic model reported by Nigam et al. (39) or in Chapter 3. The model described by Nigam et al. (39) would have predicted Entries 1, 2, and 3 to be non-nodulated and all others to be nodulated. The model described in Chapter 3 would have predicted Entries 1, 2, and 3 to be non-nodulated; Entries 10 and 12 to have few nodules; and all other entries to have normal nodulation. The only entries with normal nodulation were those that had a male parent with normal nodulation. All entries that had a non-nodulating line as the male parent were non-nodulated or had few nodules. This indicates that when the male gamete is $\underline{n_1 n_2}$ it tends to reduce the amount of nodulation, as was proposed in Chapter 3.

The F_2 data (Table 6-4) have been adjusted as described in Table 6-2. These adjustments were made assuming that the level of penetrance in these populations was the same as those discussed in

Table 6-2. Method used to adjust the F_2 nodulation data to correct for incomplete penetrance when A, B, C, D, E, and F equal the number of plants rated 0, 1, 2, 3, 4, and 5, respectively.

Cross	Nodule classification		Adjusted frequency
UF 487A x PI 445923 or PI 445924	Non-nod	=	A
	Normal	=	B + C + D + E + F
PI 262090 x PI 445923 or PI 445924	Non-nod	=	A x 0.70
	Few	=	(A x 0.30) + B + C
	Normal	=	D + E + F
Florunner x PI 445923 or PI 445924	Non-nod	=	A x 0.69
	Few	=	(A x 0.31) + (B x 0.92) + (C x 0.57)
	Normal	=	(B x 0.08) + (C x 0.43) + D + E + F

Table 6-3. Description of nodulation and testa color of F_1 plants.

Entry	Cross ♀	Cross ♂	Nodulation classification: Non-nod	Few	Normal	Testa: Color	Variegation
			no. of plants				
1	PI 445923	M4-2	6	0	0	pink	trace
2	M4-2	PI 445923	6	0	0	pink	trace
3	PI 445924	M4-2	6	0	0	light purple	trace
4	UF 487A	PI 445923	0	5	0	pink	solid
5	UF 487A	PI 445924	0	2	0	light purple	solid
6	Florunner	PI 445923	0	1	0	pink	solid
7	Florunner	PI 445924	0	2	0	light purple	solid
8	PI 445924	Florunner	0	0	1	light purple	solid
9	PI 445923	PI 262090	0	0	2	pink	trace
10	PI 262090	PI 445923	7	0	0	pink	trace
11	PI 445924	PI 262090	0	0	2	light purple	trace
12	PI 262090	PI 445924	1	0	0	light purple	trace

Table 6-4. F_2 segregation for nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the proposed model.

Entry	Cross	F_1 families		Nodule classification: Non-nod	Few	Normal	χ^2 test on adj. freq.: Source	df	χ^2	P
		no.		—— no. of plants ——						
1	UF 487A x PI 445923	5	ER‡	1	0	3	Total	5	3.20	
	$N_1N_1n_2n_2$ x $n_1n_1n_2n_2$†		O	98	29	286	Summed	1	0.36	0.50–0.75
			A	98	0	315	Homog.§	4	2.84	0.50–0.75
			E	103	0	310				
2	UF 487A x PI 445924	2	ER	1	0	3	Total	2	2.21	
	$N_1N_1n_2n_2$ x $n_1n_1n_2n_2$		O	27	8	51	Summed	1	1.87	0.10–0.25
			A	27	0	59	Homog.	1	0.34	0.50–0.75
			E	21	0	65				
3	PI 262090 x PI 445923	9	ER	1	1	2	Total	18	13.34	
	$n_1n_1N_2N_2$ x $n_1n_1n_2n_2$		O	268	94	345	Summed	2	0.90	0.50–0.75
			A	188	174	345	Homog.	16	12.44	0.50–0.75
			E	177	177	353				
4	PI 262090 x PI 445924	3	ER	1	1	2	Total	6	1.70	
	$n_1n_1N_2N_2$ x $n_1n_1n_2n_2$		O	77	30	119	Summed	2	0.64	0.50–0.75
			A	54	53	119	Homog.	4	1.06	0.75–0.90
			E	56	57	113				
5	Florunner x PI 445923	1	ER	1	1	14	Total	2	0.78	0.50–0.75
	$N_1N_1N_2N_2$ x $n_1n_1n_2n_2$		O	9	5	61				
			A	6	6	63				
			E	5	5	65				

Table 6-4.--continued.

Entry	Cross	F_1 families		Nodule classification: Non-nod	Few	Normal	χ^2 test on adj. freq.: Source	df	χ^2	P
		no.		—— no. of plants ——						
6	Florunner x PI 445924	3	ER	1	1	14	Total	6	10.43	
	$N_1N_1N_2N_2$ x $n_1n_1n_2n_2$		O	20	14	107	Summed	2	7.46	0.01-0.03
			A	14	14	113	Homog.	4	2.97	0.50-0.75
			E	9	9	123				
7	M4-2 x PI 445923	6	E	1	0	0				
	$n_1n_1n_2n_2$ x $n_1n_1n_2n_2$		O	372	0	0				
8	M4-2 x PI 445924	12	E	1	0	0				
	$n_1n_1n_2n_2$ x $n_1n_1n_2n_2$		O	897	0	0				

†Genotype of the parents.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
§Homog. = Homogeneity.

Chapter 3. A more accurate adjustment could be made if these F_2 plants were progeny tested to calculate the degree of penetrance involved when these parents are used. This method would be preferred, because it has been reported that the level of penetrance of a trait can change when the genetic background is changed (25, 35). When a chi-square test for goodness-of-fit was conducted on the adjusted frequency, Florunner x PI 445924 was the only population that was significantly different from the expected frequency. This difference could have been caused by sampling error, a different level of penetrance than reported in Chapter 3, or by a different mode of inheritance than the one proposed. Further studies are needed to determine which of these explanations is best.

Based on the model described by Nigam et al. (39), the expected frequencies in the F_2 and chi-square test would be the same as those presented in Table 6-4 for Entries 1, 2, 7, and 8. The expected ratio for Entries 3 and 4 would be 1 non-nodulated:3 nodulated, and the expected ratio for Entries 5 and 6 would be 1 non-nodulated:15 nodulated. The summed chi-square values, each having 1 df, for Entries 5, 6, 7, and 8 are 62.8, 9.92, 4.42, and 15.15, respectively. Each of these values has a probability of < 0.05. This indicates that the model reported in Chapter 3 describes the mode of inheritance of nodulation for this population better than the model reported by Nigam et al. (39).

Testa

The F_1 and F_2 data (Table 6-3, Entries 1, 2, and 10, and Table 6-5) provide evidence that pink testa color is dominant to red and supports the reports by Ashri (5, 7).

Harvey (28) found that purple was incompletely and monogenically dominant to pink and is controlled at the P locus. The F_1 and F_2 results (Table 6-3, Entries 7 and 8, and Table 6-6) also indicate that purple is incompletely dominant to pink. However, the F_2 data do not fit the 1:2:1 ratio for purple:light purple:pink that would be expected. This may have been because it is often difficult to distinguish between the purple and light purple phenotype.

The F_1 data (Table 6-3, Entries 3, 11, and 12) indicate that purple testa color is also dominant to red. The F_2 data (Table 6-7) are not significantly different from the 1 red:15 pink:48 purple ratio. This ratio can be explained by the segregation of three independent loci, P, R_2, and R_3. The P and R_2 loci have been described (5, 7, 28) but the R_3 has not. The R_3 locus has the same type of gene action as the R_2 locus with the R_3 allele producing pink testa and being dominant to the r_3 allele, which produces red testa. Additional studies are needed to substantiate the presence of the R_3 locus.

The F_1 and F_2 data (Table 6-3, Entries 1, 2, 3, 9, 10, and 12, and Table 6-8) indicate that testa variegation is incompletely dominant to solid color as previously reported (11, 12). For some F_2 plants which produced testa with trace-variegated seed, the variegated area on the seed was difficult to detect and could not be seen on all the seed. Because some plants that produced seed with trace-variegated

Table 6-5. F_2 segregation for testa color with chi-square test for goodness-of-fit to the expected ratio of 1 red:3 pink.

Cross	F_1 families		Testa color Red		Pink	χ^2 test Source	df	χ^2	P
	no.		no. of plants						
M4-2	4	O†	79	:	230	Total	4	4.75	
x		E	77	:	232	Summed	1	0.05	0.75-0.90
PI 445923						Homog.‡	3	4.70	0.10-0.25
PI 262090	6	O	133	:	415	Total	6	3.17	
x		E	137	:	411	Summed	1	0.16	0.50-0.75
PI 445923						Homog.	5	3.01	0.50-0.75

†O = Observed frequency, E = Expected frequency.
‡Homog. = Homogeneity.

Table 6-6. F_2 segregation for testa color with chi-square test for goodness-of-fit to the expected ratio of 1 pink:3 purple.

Cross	F_1 families		Testa color: Pink	:	Purple†	X^2 test: Source	df	X^2	P
	no.		no. of plants						
Florunner	3	O‡	36	:	95	Total	3	0.70	
x		E	33	:	98	Summed	1	0.43	0.50-0.70
PI 445924						Homog.§	2	0.27	0.75-0.90

†The testa that were classified as purple and light purple were grouped together to form the purple class.
‡O = Observed frequency, E = Expected frequency.
§Homog. = Homogeneity.

Table 6-7. F_2 segregation for testa color with chi-square test for goodness-of-fit to the expected ratio of 1 red:15 pink:48 purple.

Cross	F_1 families		Testa color						χ^2 test			
			Red		Pink		Purple†		Source	df	χ^2	P
	no.		—— no. of plants ——									
PI 262090	3	O‡	4	:	50	:	157		Total	6	1.91	
x		E	3	:	50	:	158		Summed	2	0.17	0.90-0.95
PI 445924									Homog.§	4	1.74	0.75-0.90
M4-2	3	O	6	:	66	:	195		Total	6	5.75	
x		E	4	:	63	:	200		Summed	2	1.13	0.50-0.75
PI 445924									Homog.	4	4.62	0.25-0.50

†The testa that were classified as purple and light purple were grouped together to form the purple class.
‡O = Observed frequency, E = Expected frequency.
§Homog. = Homogeneity.

Table 6-8. F_2 segregation for variegated testa with chi-square tests for goodness-of-fit to the expected ratio of 3 solid and trace-variegated:1 variegated.

Cross	F_1 families		Variegation† S	:	V	χ^2 test Source	df	χ^2	P
	no.		no. of plants						
M4-2	4	O‡	224	:	85	Total	4	2.66	
x		E	232	:	77	Summed	1	1.04	0.25-0.50
PI 445923						Homog.§	3	1.62	0.50-0.75
PI 262090	6	O	339	:	209	Total	6	62.20	
x		E	411	:	137	Summed	1	50.45	<0.005
PI 445923						Homog.	5	11.75	0.02-0.05
M4-2	3	O	187	:	79	Total	3	4.28	
x		E	200	:	66	Summed	1	3.13	0.05-0.10
PI 445924						Homog.	2	1.15	0.50-0.75
PI 262090	3	O	154	:	57	Total	3	3.29	
x		E	158	:	53	Summed	1	0.46	0.25-0.50
PI 445924						Homog.	2	2.83	0.10-0.25

†S = Solid and trace-variegated, V = Variegated.
‡O = Observed frequency, E = Expected frequency.
§Homog. = Homogeneity.

seed were probably classified as solid, the two classifications were combined when the F_2 data were analyzed. The F_2 segregation of PI 262090 x PI 445923 were significantly different from the expected frequency. This was probably caused by some of the heterozygotes (Vv) being classified as variegated. Further studies would need to be conducted to determine if modifier genes or some other factor may be causing the deviation from the 3:1 ratio. The other populations in Table 6-8 were not significantly different from the expected 3:1 ratio.

For testa color and variegation segregation in the F_2, both the pooled and homogeneity chi-square test had probabilities below 0.025 when all four F_1 families were tested (Table 6-9). When the family with the highest chi-square value was omitted, the probabilities were in the acceptable range. The family that was omitted had an excess number of plants with red variegated seed. This could have been caused by sampling error, but further study is needed to explain the abnormal segregation observed in this family. The results from the other three families indicate that the R_2 and V loci segregate independently, supporing the results of Branch and Hammons (12) and Chapter 5.

Table 6-10 provides evidence that the four loci P, R_2, R_3, and V segregate independently. This also supports the data presented in Table 6-9.

In Chapter 4, it was shown that the N_1 and V loci are linked. The data presented in Table 6-11 indicate that the N_2 and V loci are independent. This would be expected because N_1 and N_2 are also independent.

Table 6-9. F_2 segregation for testa color and variegation with chi-square test for goodness-of-fit to the expected ratio with independent segregation of the R_2 and V loci.

Cross	F_1 families		Pink S†	Pink V	Red S	Red V	χ^2 test Source	df	χ^2	P
	no.		no. of plants							
M4-2‡	4	ER¶	9	3	3	1	Total	12	30.75	
x		O	176	54	48	31	Pooled	3	9.09	0.02-0.05
PI 445923		E	174	58	58	19	Homog.#	9	21.66	0.01-0.03
M4-2§	3	ER	9	3	3	1	Total	9	6.81	
x		O	155	50	42	21	Pooled	3	2.55	0.25-0.50
PI 445923		E	151	50	50	17	Homog.	6	4.26	0.50-0.75

†S = Solid and trace-variegated, V = Variegated.
‡All of the F_1 families are included.
§The F_1 family with the highest chi-square value has been omitted.
¶ER = Expected ratio, O = Observed frequency, E = Expected frequency.
#Homog. = Homogeneity.

Table 6-10. F_2 segregation for testa color and variegation analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the four loci P, R_2, R_3, and V.

Cross	F_1 families		Red			Pink			Purple†			X^2 test			
			S‡		V	S		V	S		V	Source	df	X^2	P
	no.		no. of plants												
PI 262090	3	ER§	3	:	1	45	:	15	144	:	48	Total	15	11.53	
x		O	3	:	1	40	:	10	111	:	46	Pooled	5	2.38	0.75-0.90
PI 445924		E	2	:	1	37	:	12	119	:	40	Homog.¶	10	9.15	0.50-0.75
M4-2	3	ER	3	:	1	45	:	15	144	:	48	Total	15	18.05	
x		O	4	:	2	54	:	12	129	:	65	Pooled	5	10.50	0.05-0.10
PI 445924		E	3	:	1	47	:	16	150	:	50	Homog.	10	7.55	0.50-0.75

†The testa that were classified as purple and light purple were grouped together to form the purple class.
‡S = Solid and trace-variegated, V = Variegated.
§ER = Expected ratio, O = Observed frequency. E = Expected frequency.
¶Homog. = Homogeneity.

Table 6-11. F_2 segregation for testa variegation and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the V and N_2 loci.

Cross	F_1 families		Non-nod S†	Non-nod V	Few S	Few V	Normal S	Normal V	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants									
PI 262090	3	ER‡	3	1	3	1	6	2	Total	15	17.62	
x		O	53	17	18	11	83	29	Pooled	5	2.18	0.75-0.90
PI 445924		A	37	12	34	16	83	29	Homog.§	10	15.44	0.10-0.25
		E	40	13	40	13	79	26				

†S = Solid and trace-vareigated, V = Variegated.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
§Homog. = Homogeneity.

The N_2 and R_2 loci segregated independently (Table 6-12). This supports the data reported in Chapter 5 which indicate that N_1, N_2, and R_2 segregated independently. The data presented in Table 6-13 provide evidence that N_1, N_2, and P segregated independently and Table 6-14 provides evidence that P, R_2, R_3, and N_2 segregated independently.

In summary, the inheritance of nodulation in peanut was investigated using a non-nodulating line derived from the non-nodulating germplasm identified by Gorbet and Burton (24), two non-nodulating lines from ICRISAT, and three lines with normal nodulation. The results in the F_1 and F_2 generations do not fully support the mode of inheritance proposed by Nigam et al. (39) or in Chapter 3. Further studies will need to be conducted to fully elucidate the mode of inheritance of nodulation. Evidence was also presented that there is another locus, R_3, which controls testa color with pink being dominant to red. Also, the following groups of loci were found to segregate independently: (R_2, V), (P, R_2, R_3, V), (V, N_2), (R_2, N_2), (P, N_1, N_2), and (P, R_2, R_3, N_2).

Table 6-12. F_2 segregation for testa color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the N_2 and R_2 loci.

Cross	F_1 families		Non-nod Pink	Non-nod Red	Few Pink	Few Red	Normal Pink	Normal Red	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants									
PI 445923	6	ER†	3	1	3	1	6	2	Total	20	21.33	
x		O	158	55	48	19	209	59	Pooled	5	3.09	0.50-0.75
PI 262090		A	111	38	95	36	209	59	Homog.‡	25	18.24	0.75-0.90
		E	103	34	103	34	206	68				

†ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
‡Homog. = Homogeneity.

Table 6-13. F_2 segregation for testa color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the $\underline{N}_1$, $\underline{N}_2$, and $\underline{P}$ loci.

Cross	F_1 families		Non-nod Purple†	Non-nod Pink	Few Purple	Few Pink	Normal Purple	Normal Pink	χ^2 test on adj. freq. Source	df	χ^2	P
	no.		no. of plants									
Florunner	3	ER‡	3	1	3	1	42	14	Total	15	15.12	
x		O	12	6	11	3	72	27	Pooled	5	7.95	0.10-0.25
PI 445924		A	8	4	10	4	76	28	Homog.§	10	7.17	0.50-0.75
		E	6	2	6	2	86	29				

†The testa that were classified as purple and light purple were grouped together to form the purple class.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
§Homog. = Homogeneity.

Table 6-14. F_2 segregation for testa color and nodulation with the adjusted frequency analyzed by chi-square test for goodness-of-fit to the expected ratio with independent segregation of the P, R_2, R_3, and N_2 loci.

Cross	F_1 families		Non-nod			Few			Normal			χ^2 test on adj. freq.		
			Red	Pink	Purple†	Red	Pink	Purple	Red	Pink	Purple	Source	df	χ^2	P
	no.		no. of plants												
PI 262090	3	ER‡	1	15	48	1	15	48	2	30	96	Total	24	13.32	
x		O	2	12	56	0	8	21	2	30	80	Pooled	8	3.07	0.90-0.95
PI 445924		A	1	8	39	1	12	38	2	30	80	Homog.§	16	10.25	0.75-0.90
		E	1	12	40	1	12	40	2	25	79				

†The testa that were classified as purple and light purple were grouped together to form the purple class.
‡ER = Expected ratio, O = Observed frequency, A = Adjusted frequency, E = Expected frequency.
§Homog. = Homogeneity.

CHAPTER 7
SUMMARY AND CONCLUSIONS

The inheritance of nodulation and its association with genes controlling testa color were investigated on peanut (Arachis hypogaea L.). A diallel cross was made using M4-2, a non-nodulating line, and three nodulating peanut lines, PI 262090, UF 487A, and 'Florunner.' Selected F_1 plants were backcrossed to M4-2 or PI 262090. The F_1, F_2, F_3, F_1BC_1, and F_2BC_1 generations were field grown at the University of Florida Agricultural Research Center, Marianna, Florida. Nodulation classifications were determined by observing plants and rating roots on each plant from 0 (no nodules) through 5 (abundant nodules). Pod samples were taken and testa color was evaluated. These data were analyzed by chi-square test for goodness-of-fit to the proposed model.

The results indicate that inheritance of nodulation is controlled at two loci, $\underline{N_1}$ and $\underline{N_2}$. The non-nodulating genotype (M4-2) is $\underline{n_1n_1n_2n_2}$, and all other genotypes have normal nodulation, except $\underline{n_1n_1N_2n_2}$, which had few nodules when the parental male gamete was $\underline{n_1n_2}$. The locus controlling testa variegation, $\underline{V}$, was found to be linked to $\underline{N_1}$ with an average crossover rate of about 10%. Testa variegation color is controlled at one locus. The allele causing white variegation, $\underline{Wv}$, is dominant to the allele causing tinted variegation, $\underline{wv}$. The $\underline{N_2}$ and $\underline{Wv}$ loci segregated independently. The $\underline{R_2}$ locus, which controls red and pink testa color, segregated independently from the $\underline{V}$ locus. It was also determined that the $\underline{N_1}$, $\underline{N_2}$, and $\underline{R_2}$ loci segregate independently.

In another study, two non-nodulating peanut lines, PI 445923 and PI 445924, were crossed with M4-2, PI 262090, UF 487A, and Florunner. Data were collected from the F_1 and F_2 generations for nodulation and testa color. The results do not fully support the model for inheritance of nodulation described in the first study. The allele causing purple testa color, P, appeared to be dominant to pink and red. There also appeared to be a duplicate locus of R_2 which was designated R_3. The following groups of loci were found to segregate independently: (R_2, V), (P, R_2, R_3, V), (V, N_2), (R_2, N_2), (P, N_1, N_2), and (P, R_2, R_3, N_2).

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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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